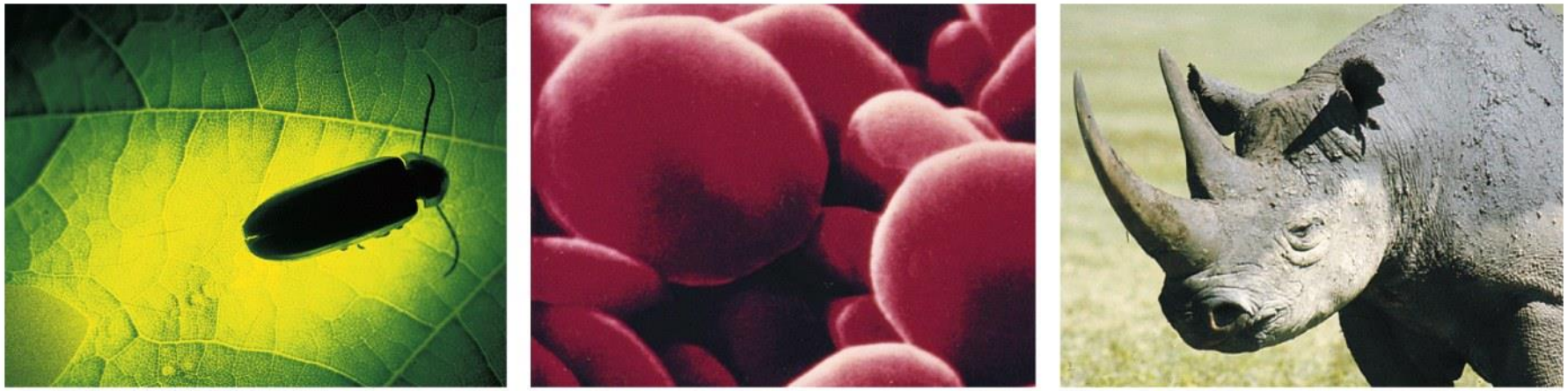
# Chapter 3
# Amino Acids, Peptides, and Proteins

# 1. Proteins are the main agents of biological function

- **The most abundant biological macromolecules in all cells**
- **Occur in great diversity with different properties and activities**
- **Mediate almost all the biological processes in a cell**
- **Constructed from 20 amino acids**

# Biological function of proteins

- **Catalysis:**
  - **hexokinase (in the glycolytic pathway)**
  - **DNA polymerase (in DNA replication)**
- **Transport:**
  - **hemoglobin (transports $O_2$ in the blood)**
  - **lactose permease (transports lactose across cell membrane)**
- **Structure:**
  - **collagen (connective tissue)**
  - **keratin (hair, nails, feathers, horns)**
- **Motion:**
  - **myosin (muscle tissue)**
  - **actin (muscle tissue, cell motility)**



(a) (b) (c)

**Figure 3-1**
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# Biological functions of proteins

# 2. Amino acids: building blocks of proteins

- **Proteins are heteropolymers of α-amino acids**
- **Amino acids varies in size, shape, charge, hydrogen-bonding capacity, hydrophobic character, and chemical reactivity**
- **Amino acids have properties that are well suited to carry out a variety of biological functions:**
  - **capacity to polymerize**
  - **useful acid-base properties**
  - **varied physical properties**
  - **varied chemical functionality**

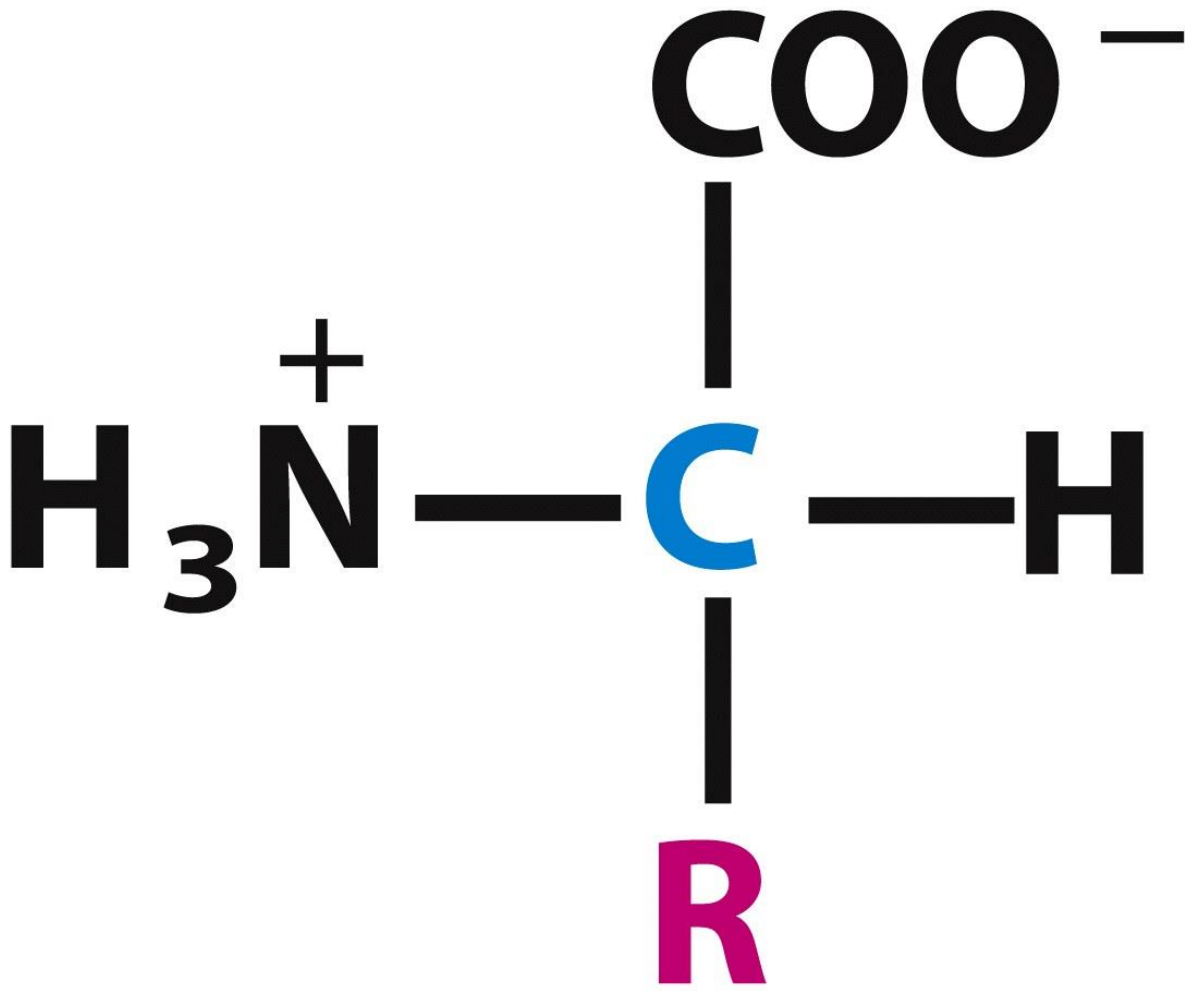


**Figure 3-2**
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# General structure of an amino acid

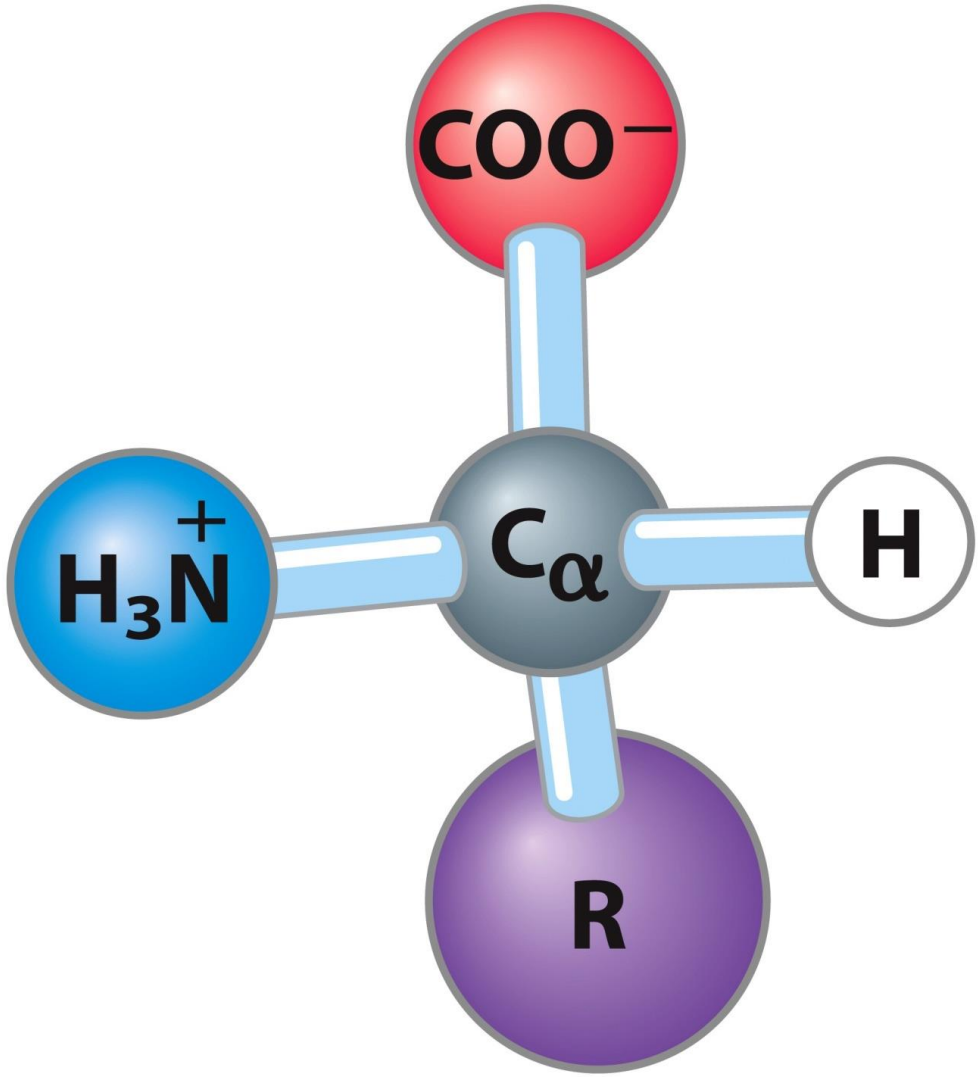


**Figure 3-2**
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# General structure of an amino acid

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**TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins**

| Amino acid | Abbreviation/ symbol | $M_r$* | p$K_a$ values: p$K_1$ (—COOH) | p$K_2$ ($—NH_3^+$) | p$K_R$ (R group) | pI | Hydropathy index† | Occurrence in proteins (%)‡ |
|---|---|---|---|---|---|---|---|---|
| **Nonpolar, aliphatic** | | | | | | | | |
| **R groups** | | | | | | | | |
| Glycine | Gly G | 75 | 2.34 | 9.60 | | 5.97 | −0.4 | 7.2 |
| Alanine | Ala A | 89 | 2.34 | 9.69 | | 6.01 | 1.8 | 7.8 |
| Proline | Pro P | 115 | 1.99 | 10.96 | | 6.48 | 1.6 | 5.2 |
| Valine | Val V | 117 | 2.32 | 9.62 | | 5.97 | 4.2 | 6.6 |
| Leucine | Leu L | 131 | 2.36 | 9.60 | | 5.98 | 3.8 | 9.1 |
| Isoleucine | Ile I | 131 | 2.36 | 9.68 | | 6.02 | 4.5 | 5.3 |
| Methionine | Met M | 149 | 2.28 | 9.21 | | 5.74 | 1.9 | 2.3 |
| **Aromatic** | | | | | | | | |
| **R groups** | | | | | | | | |
| Phenylalanine | Phe F | 165 | 1.83 | 9.13 | | 5.48 | 2.8 | 3.9 |
| Tyrosine | Tyr Y | 181 | 2.20 | 9.11 | 10.07 | 5.66 | −1.3 | 3.2 |
| Tryptophan | Trp W | 204 | 2.38 | 9.39 | | 5.89 | −0.9 | 1.4 |
| **Polar, uncharged** | | | | | | | | |
| **R groups** | | | | | | | | |
| Serine | Ser S | 105 | 2.21 | 9.15 | | 5.68 | −0.8 | 6.8 |
| Threonine | Thr T | 119 | 2.11 | 9.62 | | 5.87 | −0.7 | 5.9 |
| Cysteine§ | Cys C | 121 | 1.96 | 10.28 | 8.18 | 5.07 | 2.5 | 1.9 |
| Asparagine | Asn N | 132 | 2.02 | 8.80 | | 5.41 | −3.5 | 4.3 |
| Glutamine | Gln Q | 146 | 2.17 | 9.13 | | 5.65 | −3.5 | 4.2 |
| **Positively charged** | | | | | | | | |
| **R groups** | | | | | | | | |
| Lysine | Lys K | 146 | 2.18 | 8.95 | 10.53 | 9.74 | −3.9 | 5.9 |
| Histidine | His H | 155 | 1.82 | 9.17 | 6.00 | 7.59 | −3.2 | 2.3 |
| Arginine | Arg R | 174 | 2.17 | 9.04 | 12.48 | 10.76 | −4.5 | 5.1 |
| **Negatively charged** | | | | | | | | |
| **R groups** | | | | | | | | |
| Aspartate | Asp D | 133 | 1.88 | 9.60 | 3.65 | 2.77 | −3.5 | 5.3 |
| Glutamate | Glu E | 147 | 2.19 | 9.67 | 4.25 | 3.22 | −3.5 | 6.3 |

*$M_r$ values reflect the structures as shown in Figure 3–5. The elements of water ($M_r$ 18) are deleted when the amino acid is incorporated into a polypeptide.

†A scale combining hydrophobicity and hydrophilicity of R groups. The values reflect the free energy ($\Delta G$) of transfer of the amino acid side chain from a hydrophobic solvent to water. This transfer is favorable ($\Delta G < 0$; negative value in the index) for charged or polar amino acid side chains, and unfavorable ($\Delta G > 0$; positive value in the index) for amino acids with nonpolar or more hydrophobic side chains. See Chapter 11. From Kyte, J. & Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105–132.

‡Average occurrence in more than 1,150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed.), pp. 599–623, Plenum Press, New York.

§Cysteine is generally classified as polar despite having a positive hydropathy index. This reflects the ability of the sulfhydryl group to act as a weak acid and to form a weak hydrogen bond with oxygen or nitrogen.

**Table 3-1**

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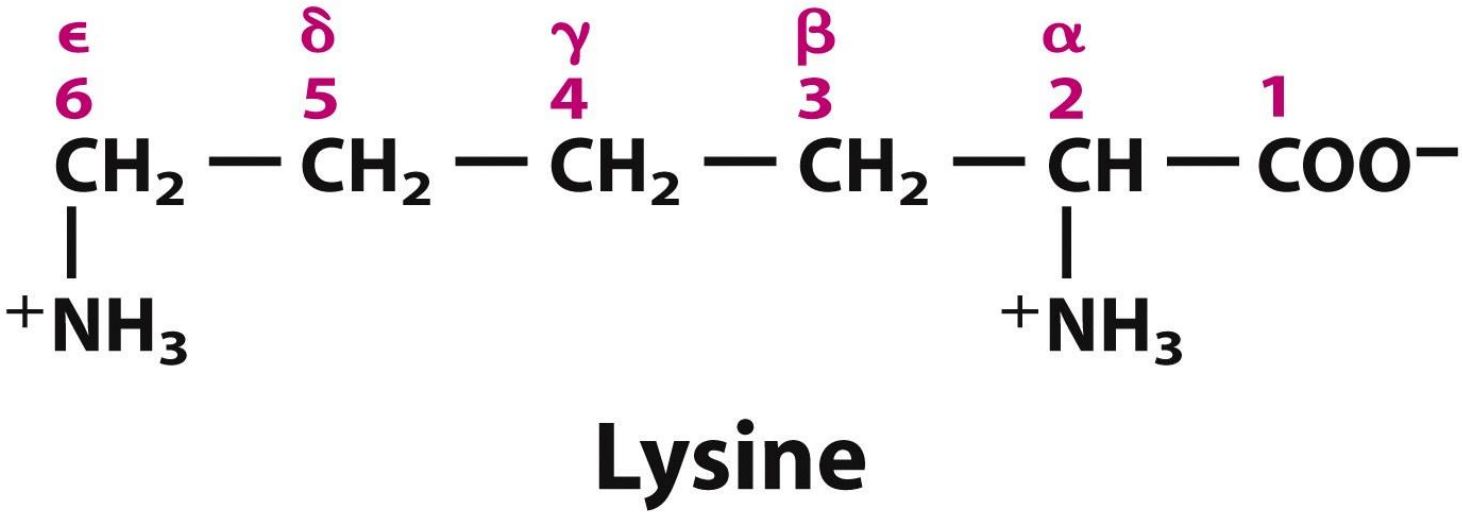
# Amino acids: atom naming

- **Organic nomenclature: start from one end**
- **Biochemical designation: start from α-carbon and go down the R-group**




Lysine

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# Most α-amino acids are chiral

- **The α-carbon has four substituents and is tetrahedral (except glycine)**
- **All (except proline) have an acidic carboxyl group, a basic amino group, and an alpha hydrogen connected to the α-carbon**
- **Each amino acid has a unique fourth substituent R group**
- **In glycine, the fourth substituent is also hydrogen**

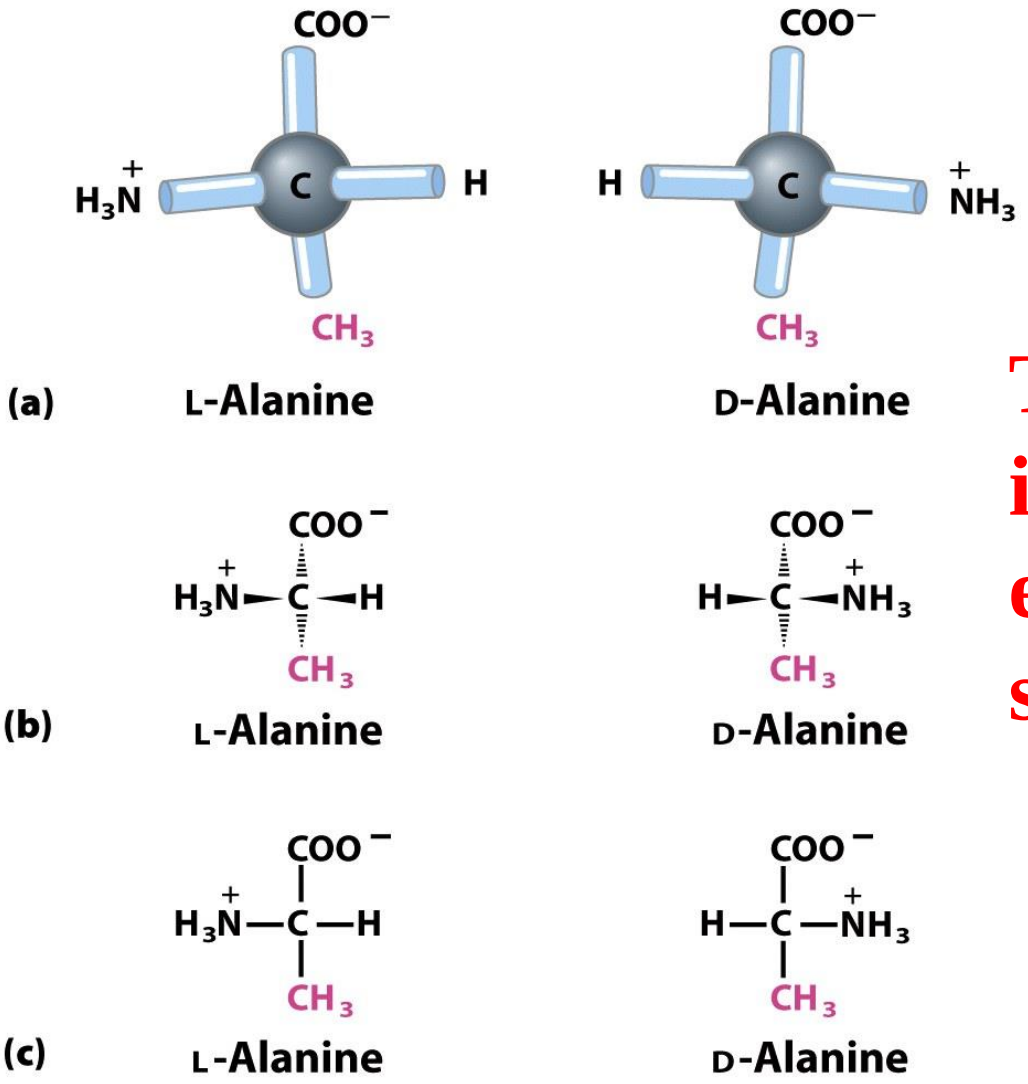


Figure 3-3
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**The amino acids in proteins are exclusively L stereoisomers.**

# Stereoisomerism in α-amino acids

# Amino acids: classification

**Common amino acids can be classified into five main groups depending on their R groups:**

- **Nonpolar, aliphatic (7)**
- **Aromatic (3)**
- **Polar, uncharged (5)**
- **Positively charged (3)**
- **Negatively charged (2)**

# Nonpolar, aliphatic R groups

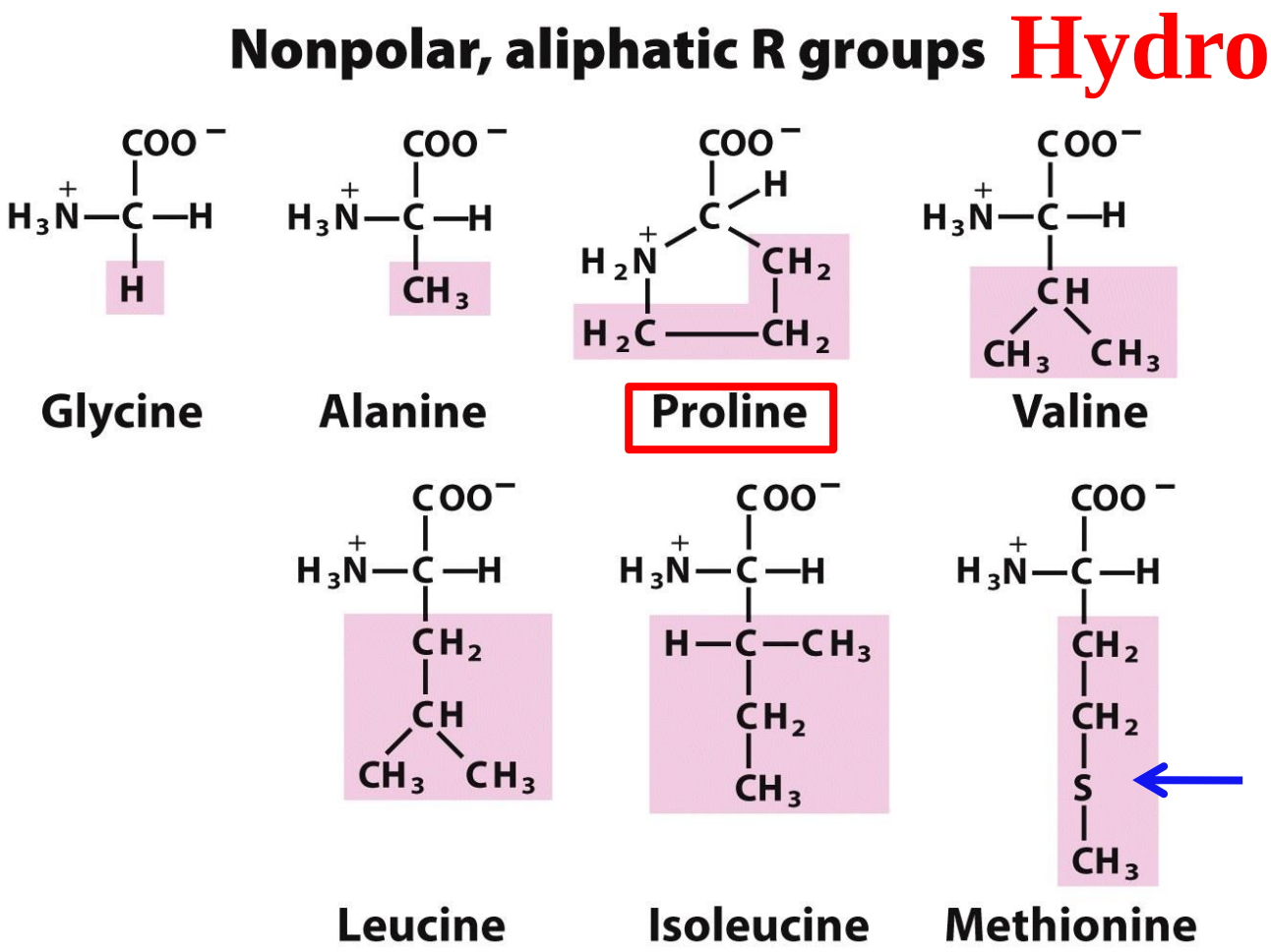


**Figure 3-5 part 1**
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# Aromatic R groups

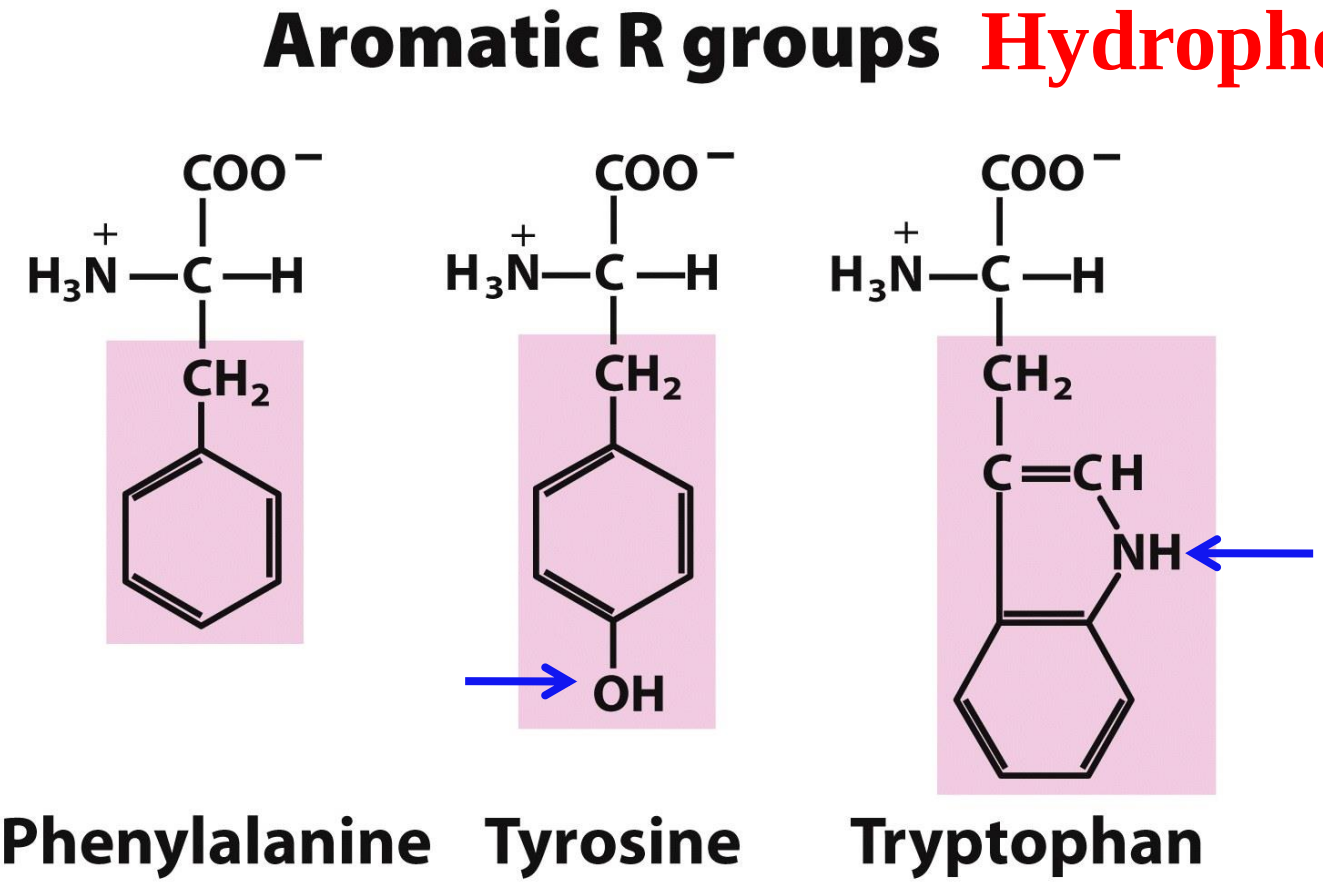


Figure 3-5 part 2
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**These amino acid side chains absorb UV light at 270-280 nm**

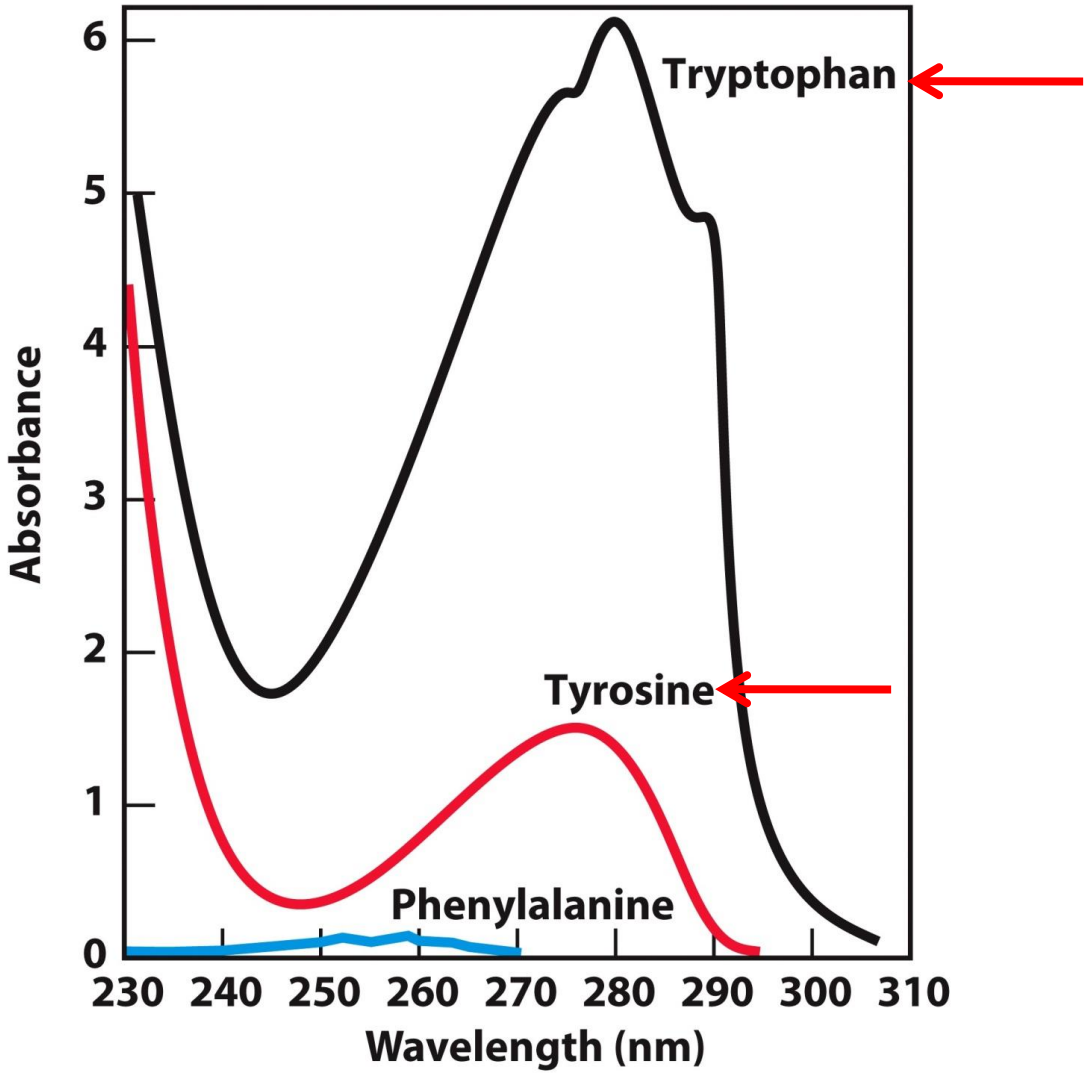


**Figure 3-6**
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# Absorption of ultraviolet light by aromatic amino acids

# Positively charged (basic) R groups

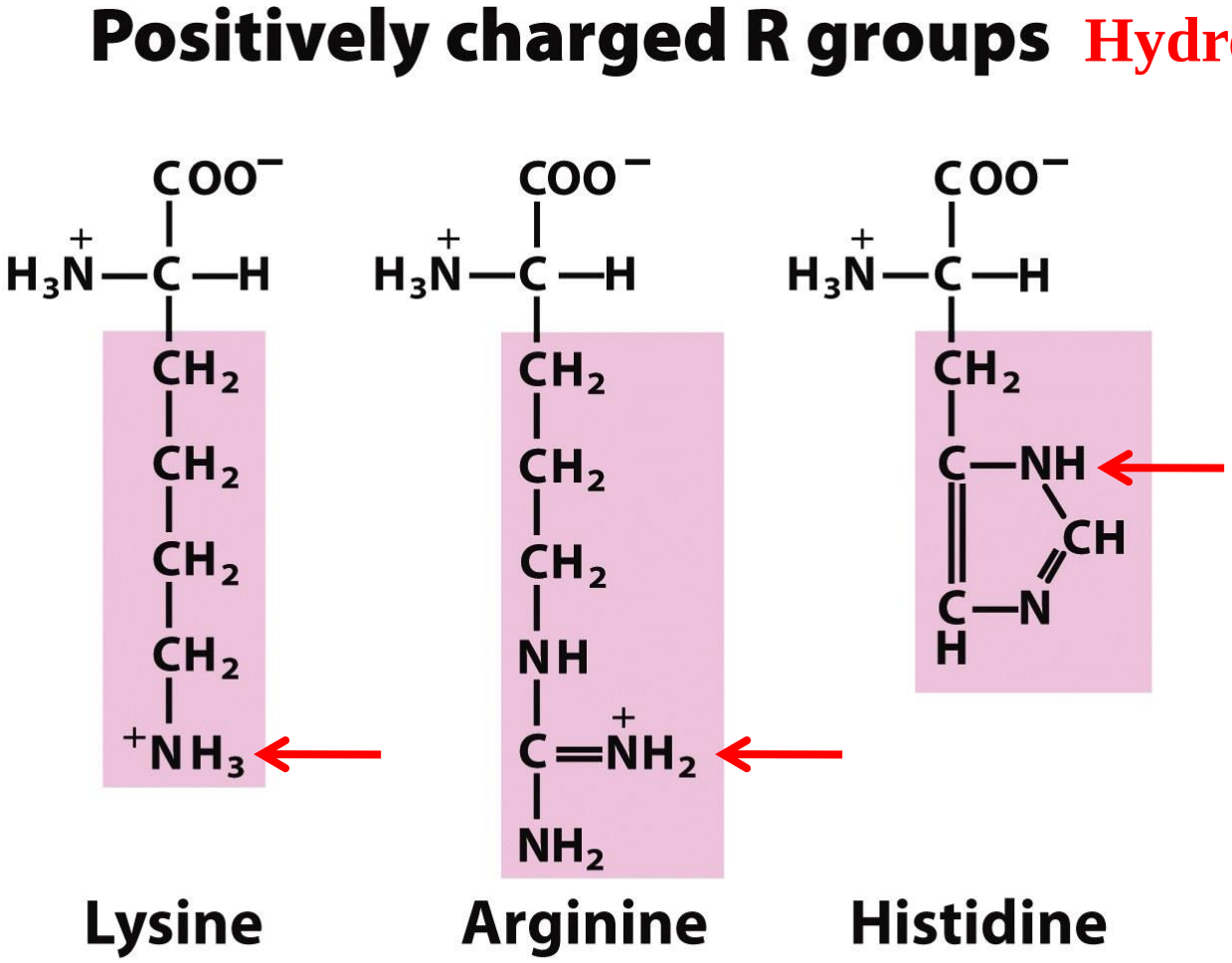


**Figure 3-5 part 4**
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# Negatively charged (acidic) R groups

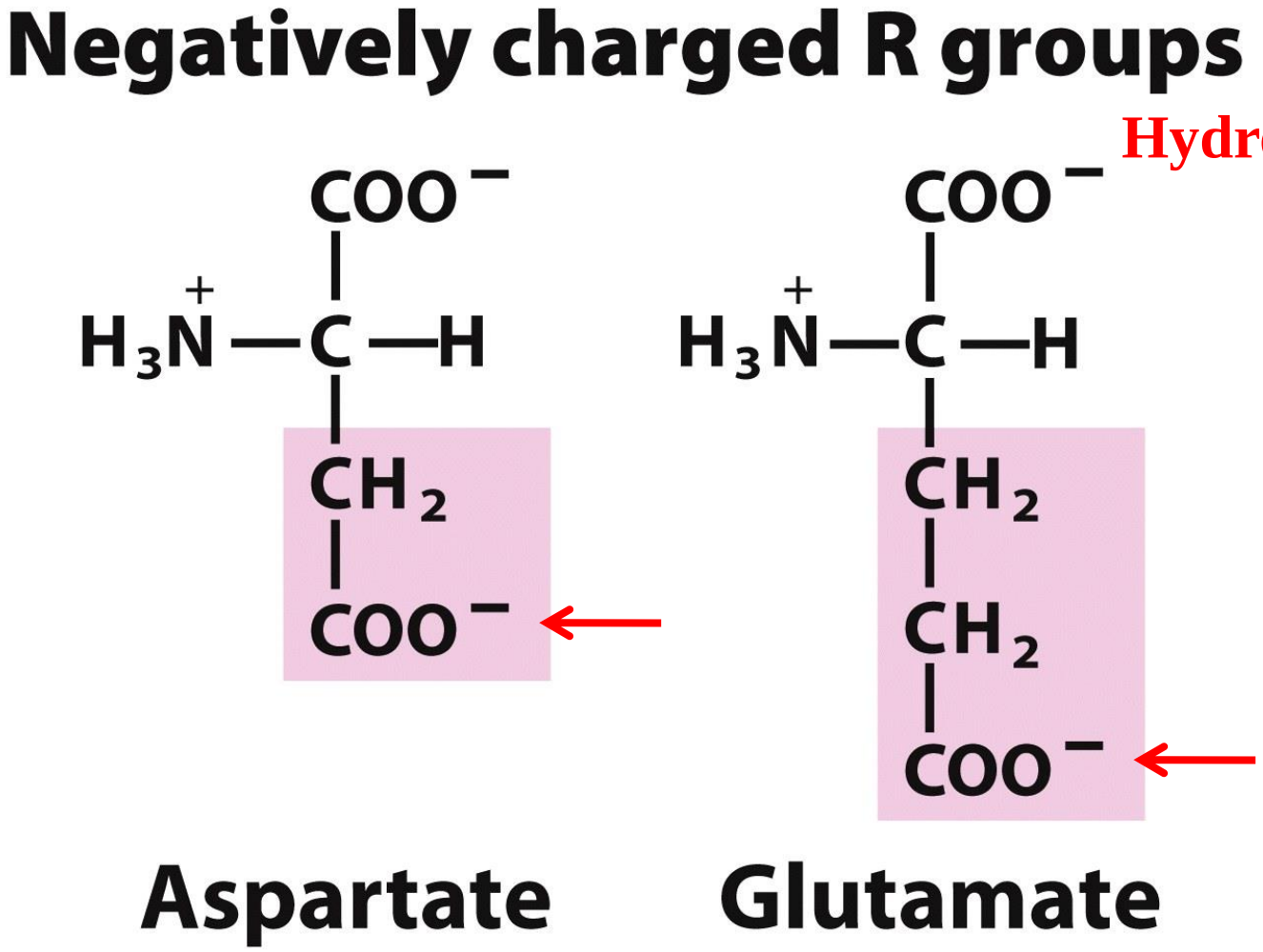


Figure 3-5 part 5
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# Polar, uncharged R groups

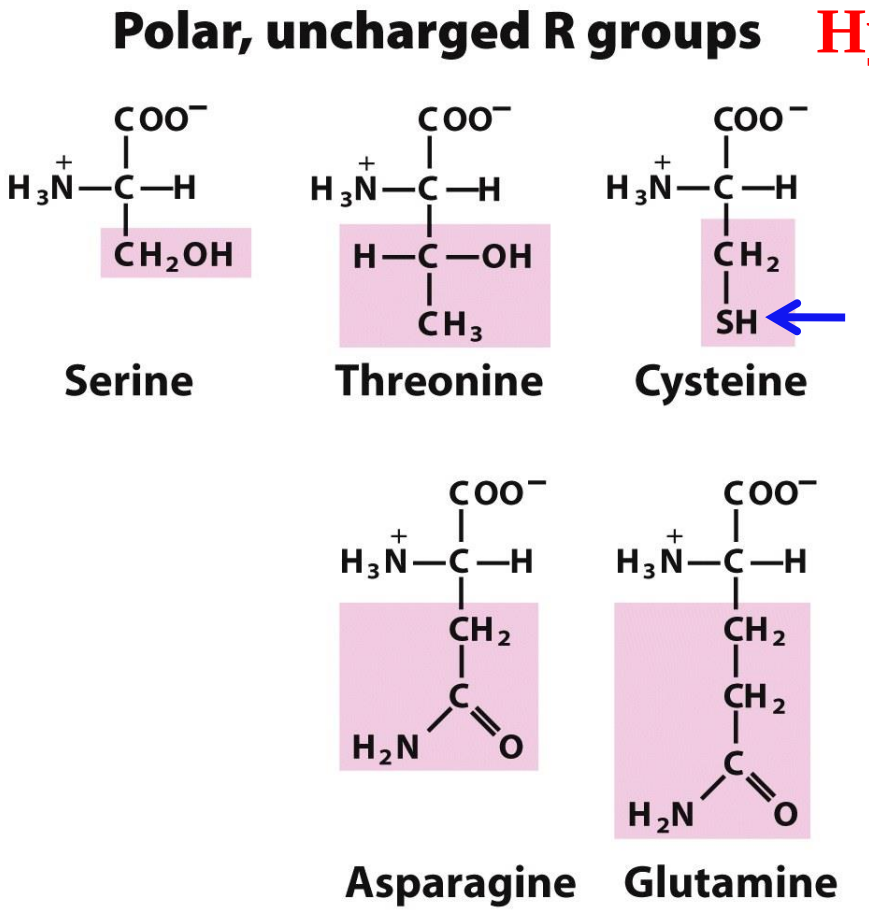


Figure 3-5 part 3
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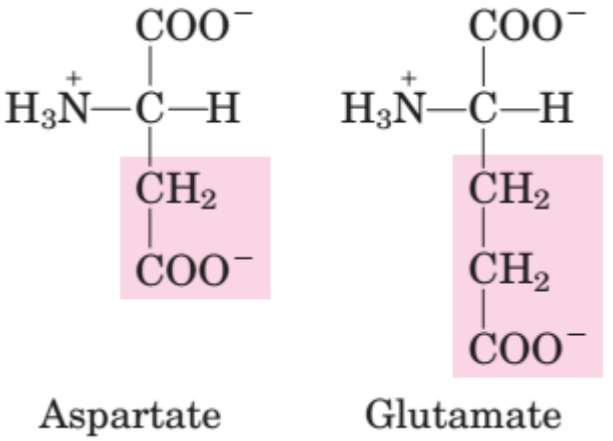
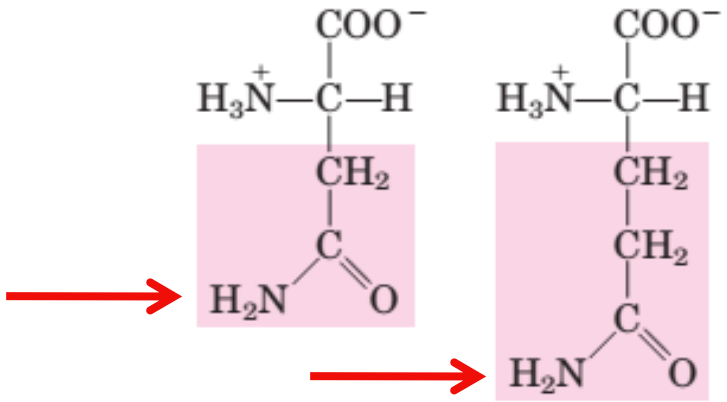
**These amino acids side chains can form hydrogen bonds.**

# Asn and Gln are the amides of Asp and Glu




Aspartate Glutamate




Asparagine Glutamine

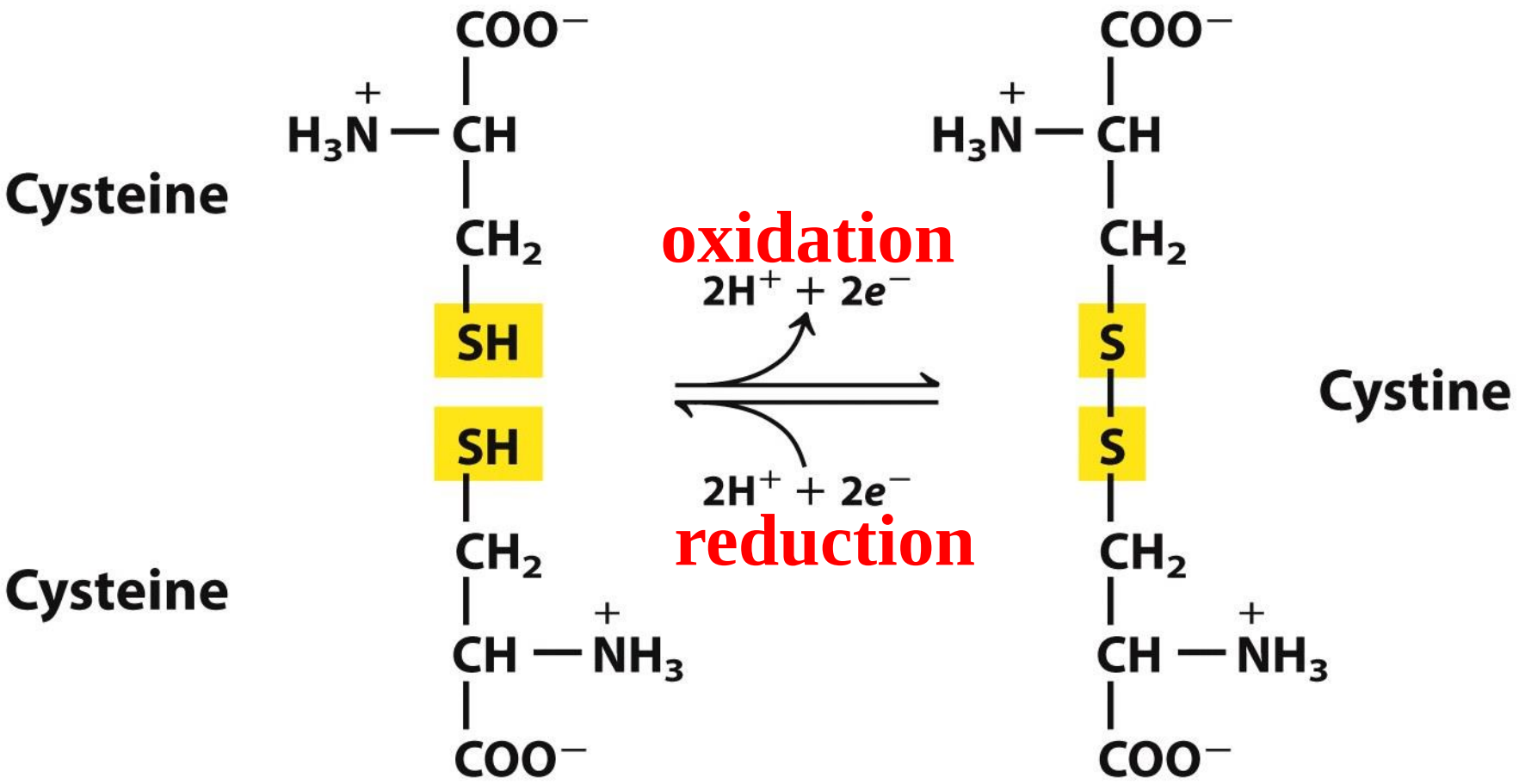



**Figure 3-7**
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**Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine**

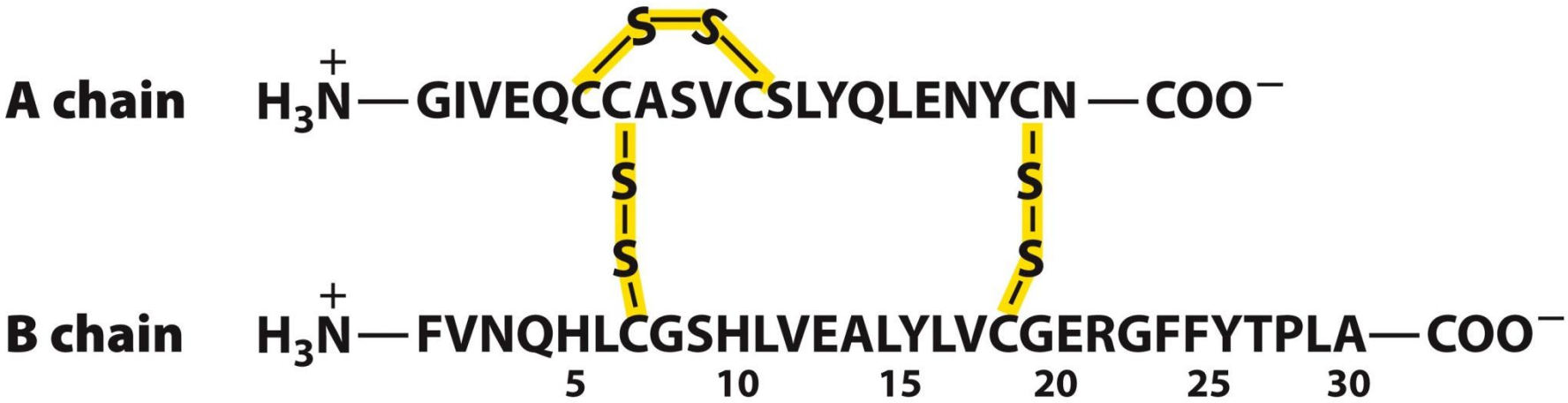


**Figure 3-24**
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# Amino acid sequence of bovine insulin

**The two polypeptide chains of insulin are joined by disulfide cross-linkages.**

# Uncommon amino acids in proteins

- **Not incorporated by ribosomes**
- **Derived from common amino acids by post-translational modification**
- **Reversible modifications, for example, phosphorylation, are important in regulation and signaling**

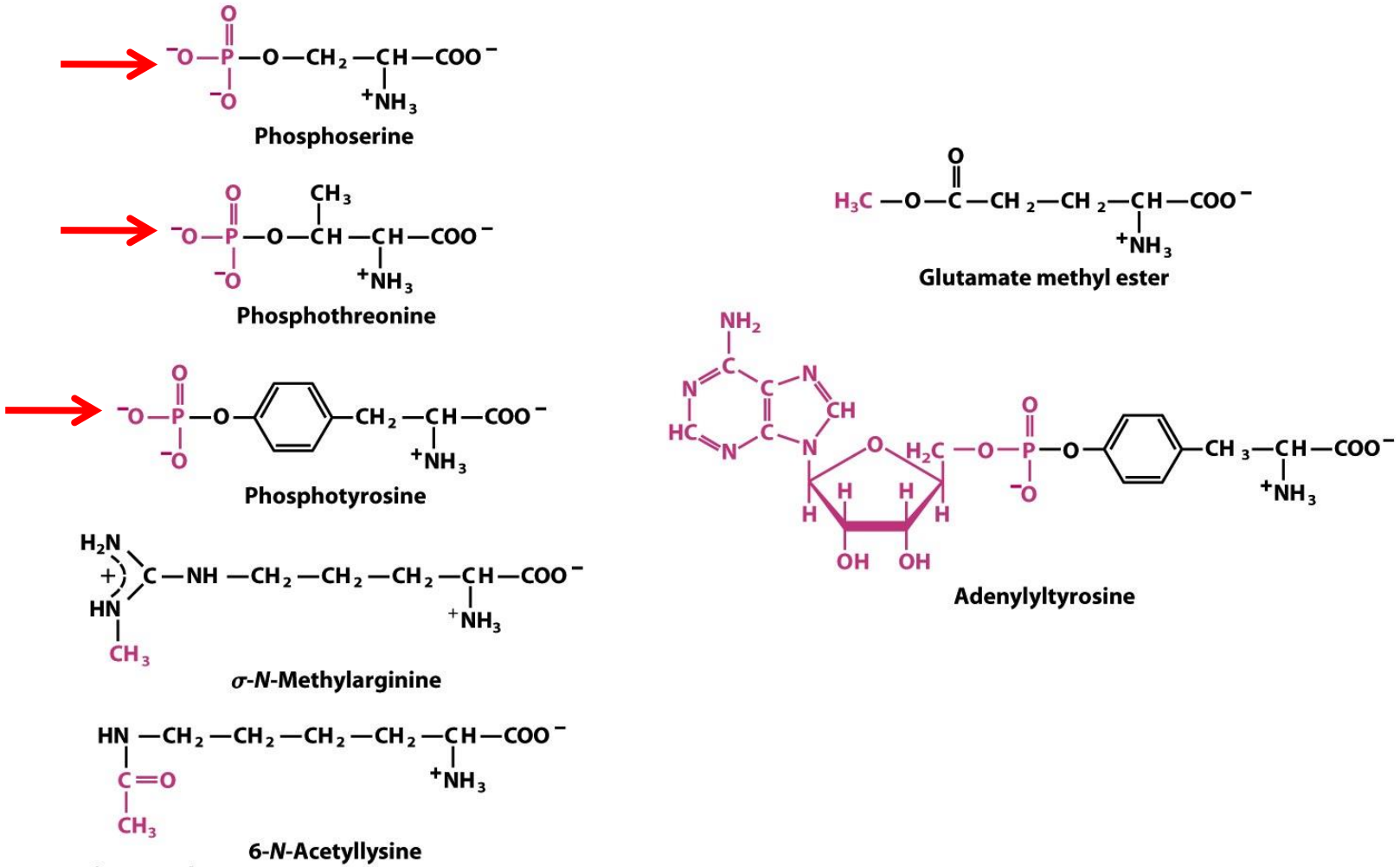


Figure 3-8b
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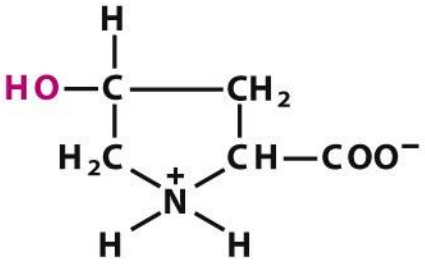
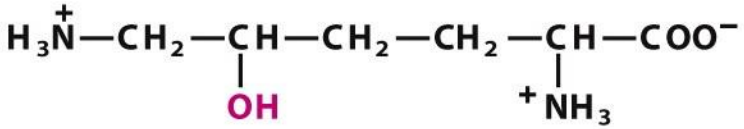
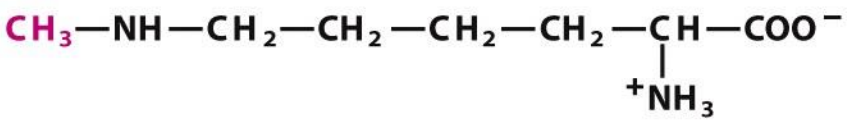
# Reversible amino acid modifications involved in regulation of protein activity



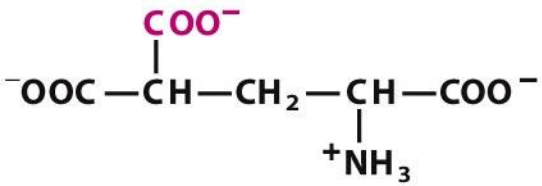
4-Hydroxyproline



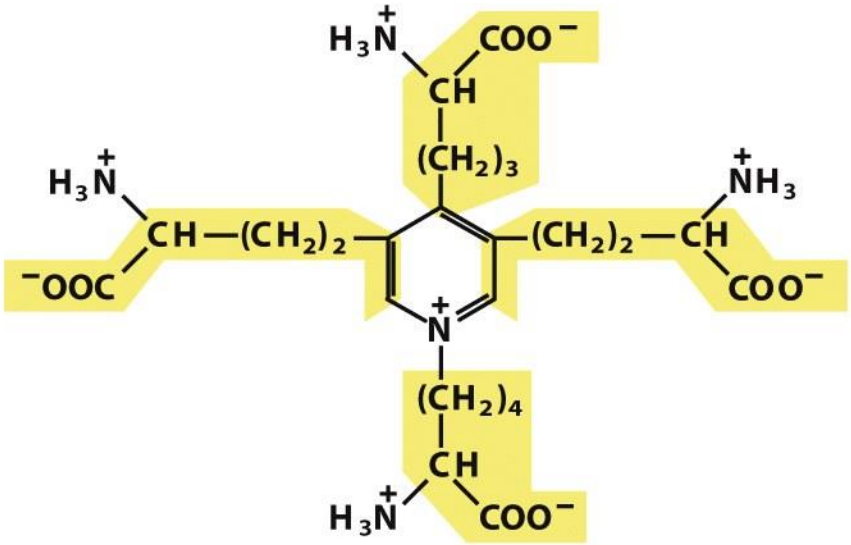
5-Hydroxylysine



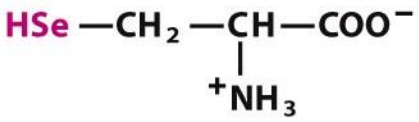
6-*N*-Methyllysine



γ-Carboxyglutamate



Desmosine



Selenocysteine

**Figure 3-8a**

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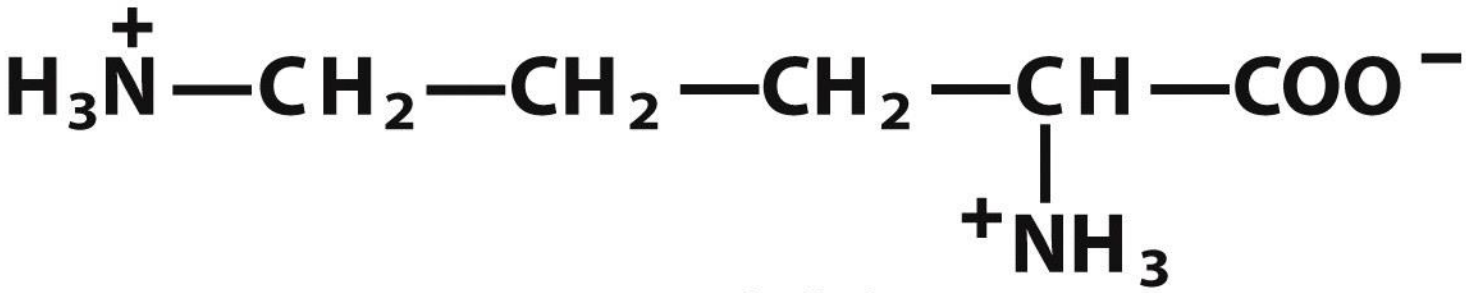
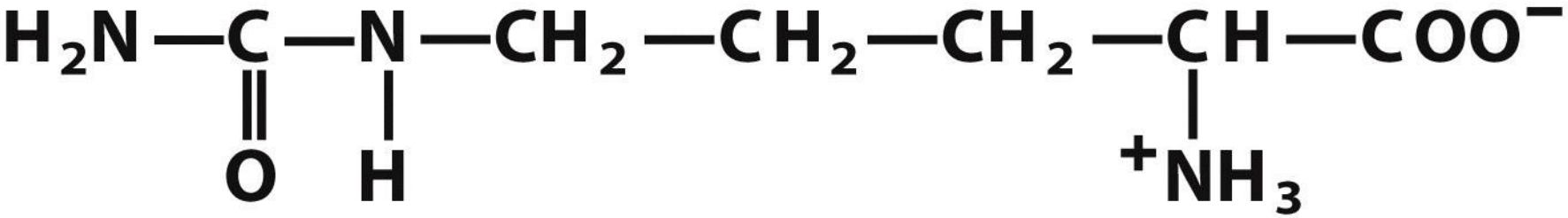
# Uncommon amino acids



Ornithine



Citrulline

**Figure 3-8c**
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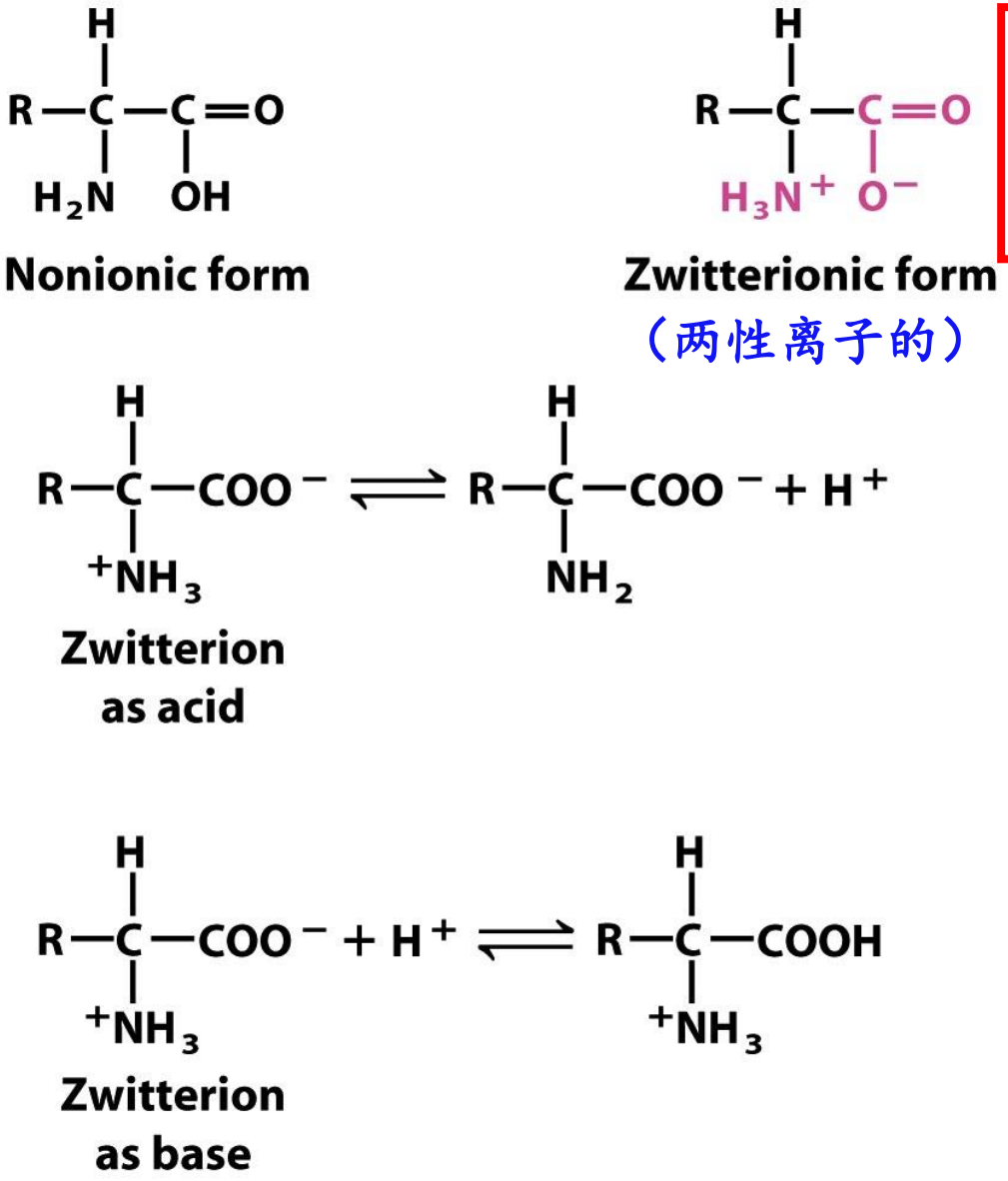
**Ornithine and citrulline, not found in proteins, are intermediates in the biosynthesis of arginine and in the urea cycle.**

# Ionization of amino acids

- **The carboxyl, the amino and the R groups of some amino acids function as weak acids and bases**
- **At acidic pH, the carboxyl group is protonated and the amino acid is in the cationic form**
- **At neutral pH, the carboxyl group is deprotonated but the amino group is protonated. The net charge is zero; such ions are called Zwitterions**
- **At alkaline pH, the amino group is neutral ($-NH_2$) and the amino acid is in the anionic form**




**Dipolar ions, predominates at neutral pH**

Figure 3-9
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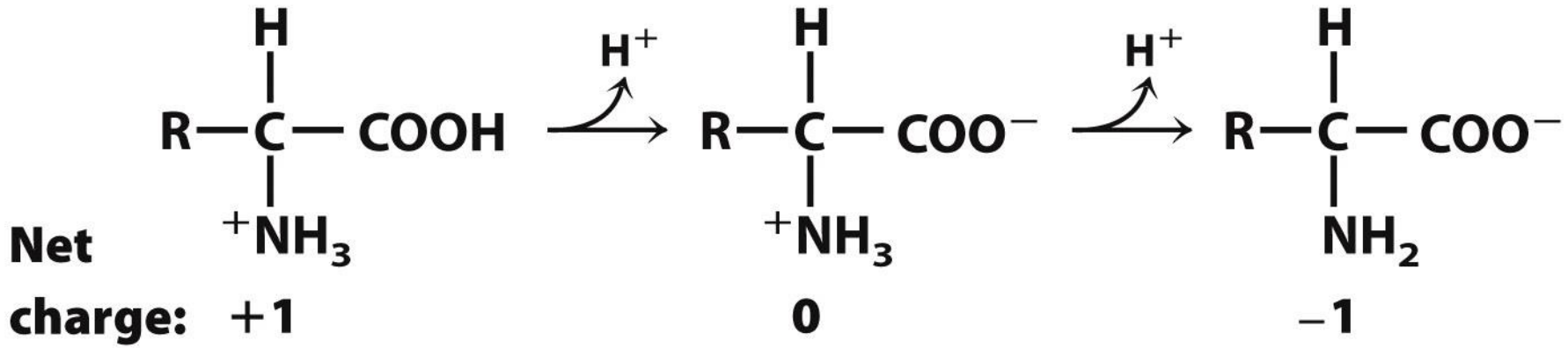
# Nonionic and zwitterionic forms of amino acids

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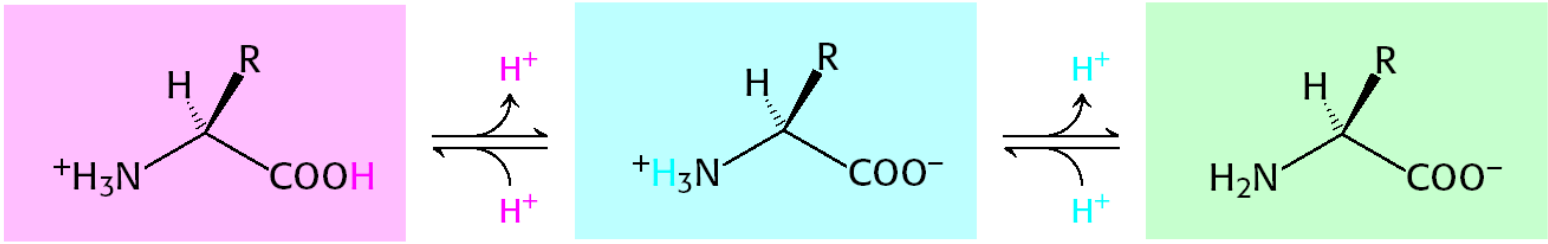
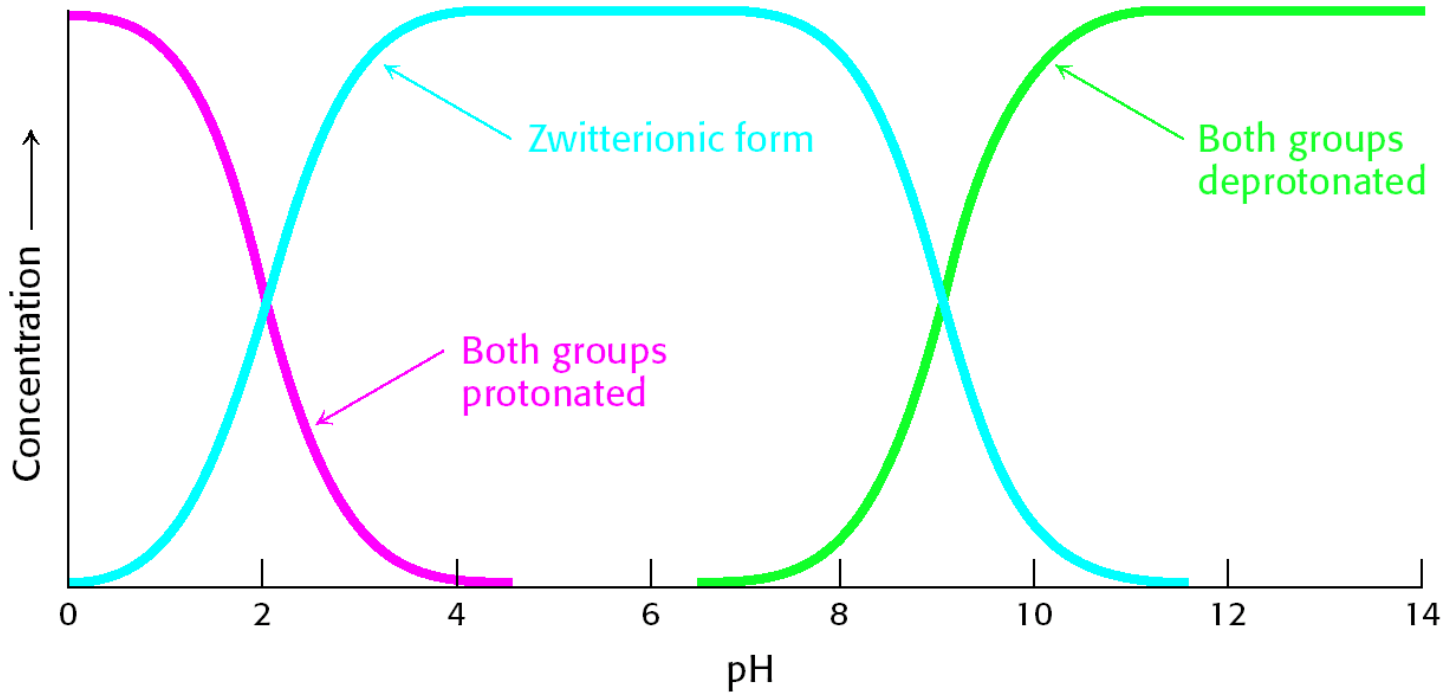
**Both the amino and the carboxyl group of an amino acid can yield protons --- diprotic**




# Ionization state as a function of pH

**The ionization state of amino acids is altered by a change in pH. The zwitterionic form predominates near physiological pH.**

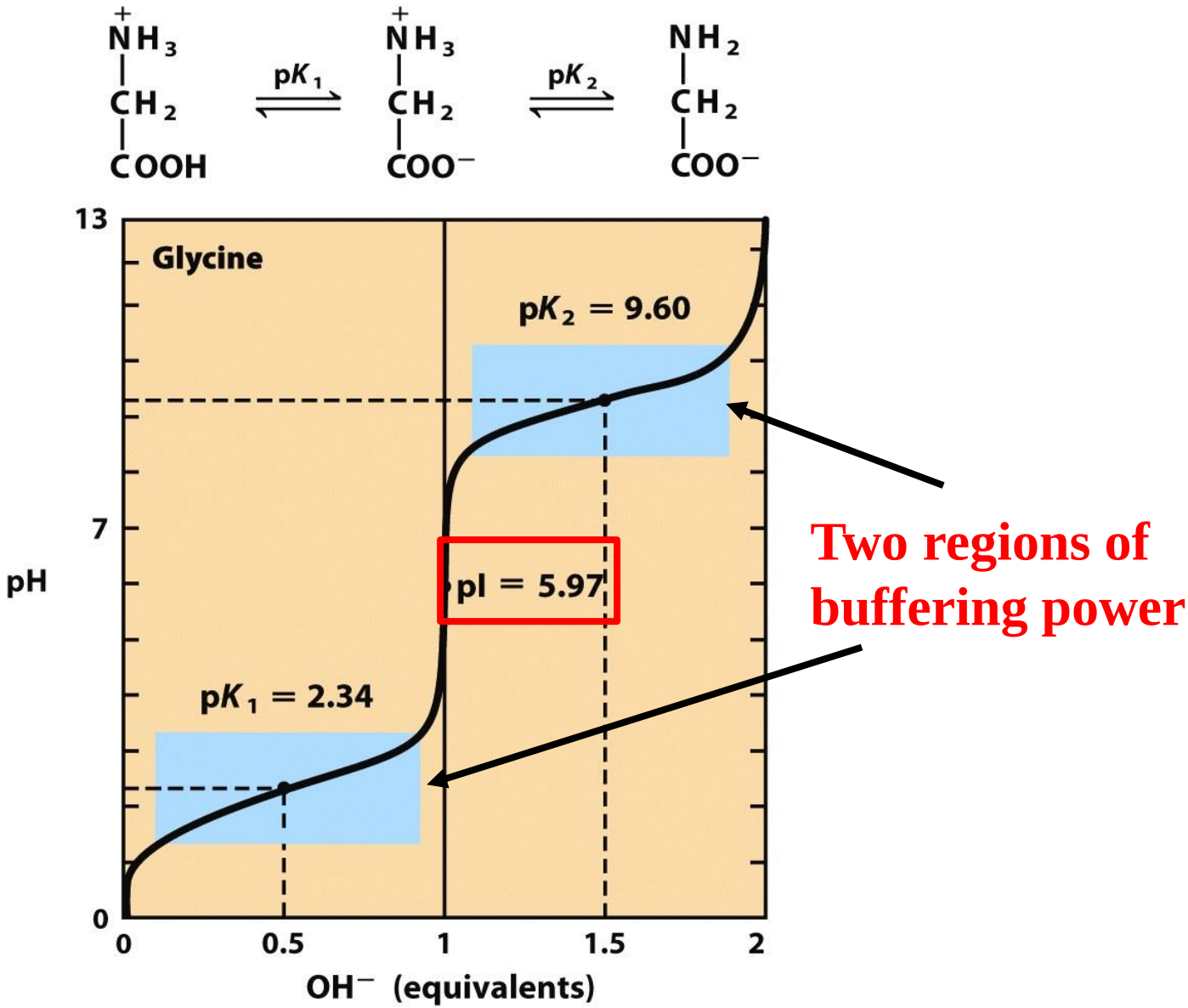



**Figure 3-10**
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# Titration curve of glycine

# Amino acids can act as buffers

- **Amino acids with uncharged side-chains, such as glycine, have two p$K_a$ values**
- **The p$K_a$ of the $\alpha$-carboxyl group of glycine is 2.34**
- **The p$K_a$ of the $\alpha$-amino group of glycine is 9.6**
- **Glycine can act as a buffer in two pH regions.**

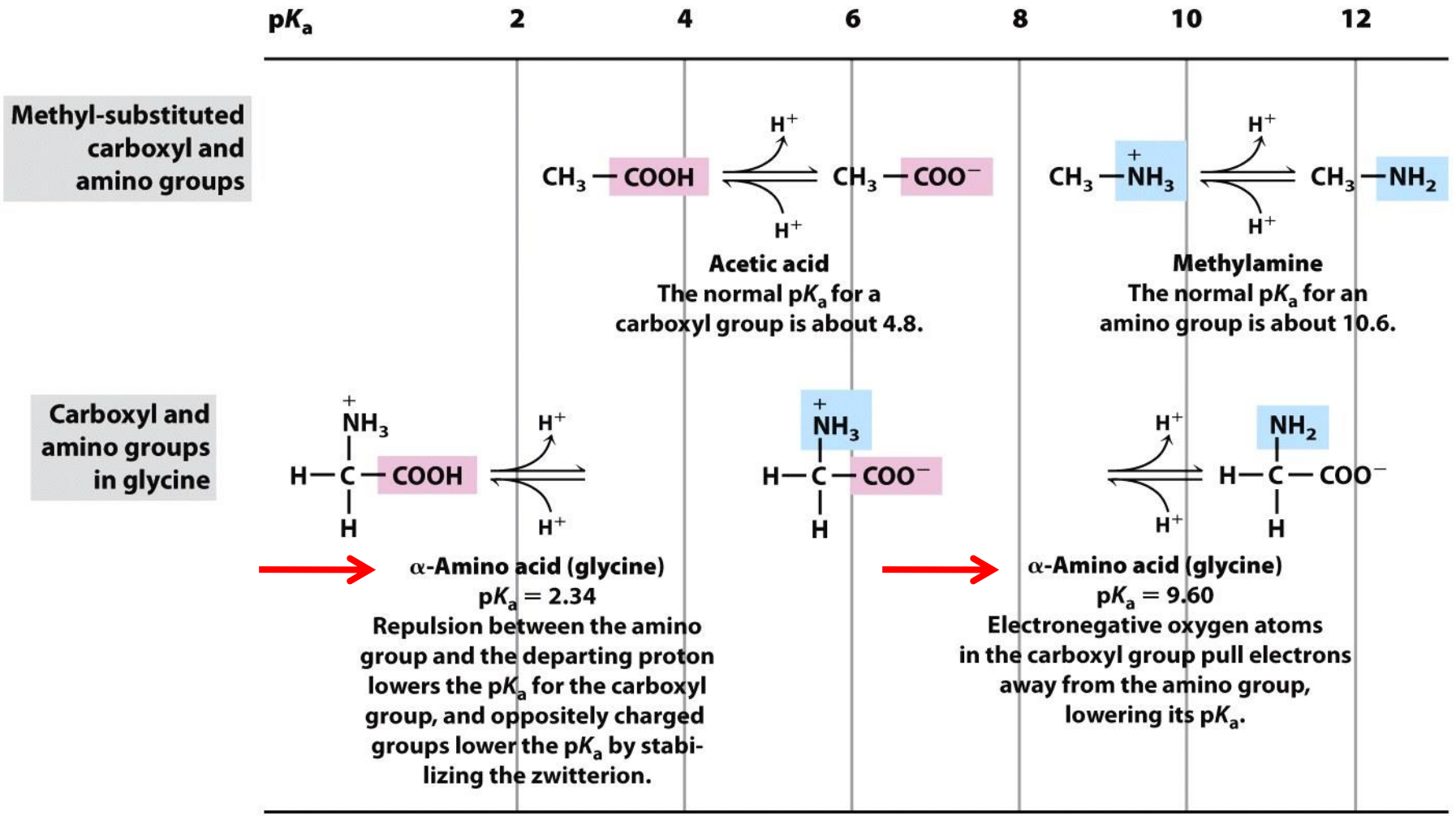


**Figure 3-11**
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# Effect of the chemical environment on p$K_a$

**α-carboxyl group is much more acidic than in carboxylic acids, whereas α-amino group is slightly less basic than in amines.**

# Amino acids carry a net charge of zero at a specific pH

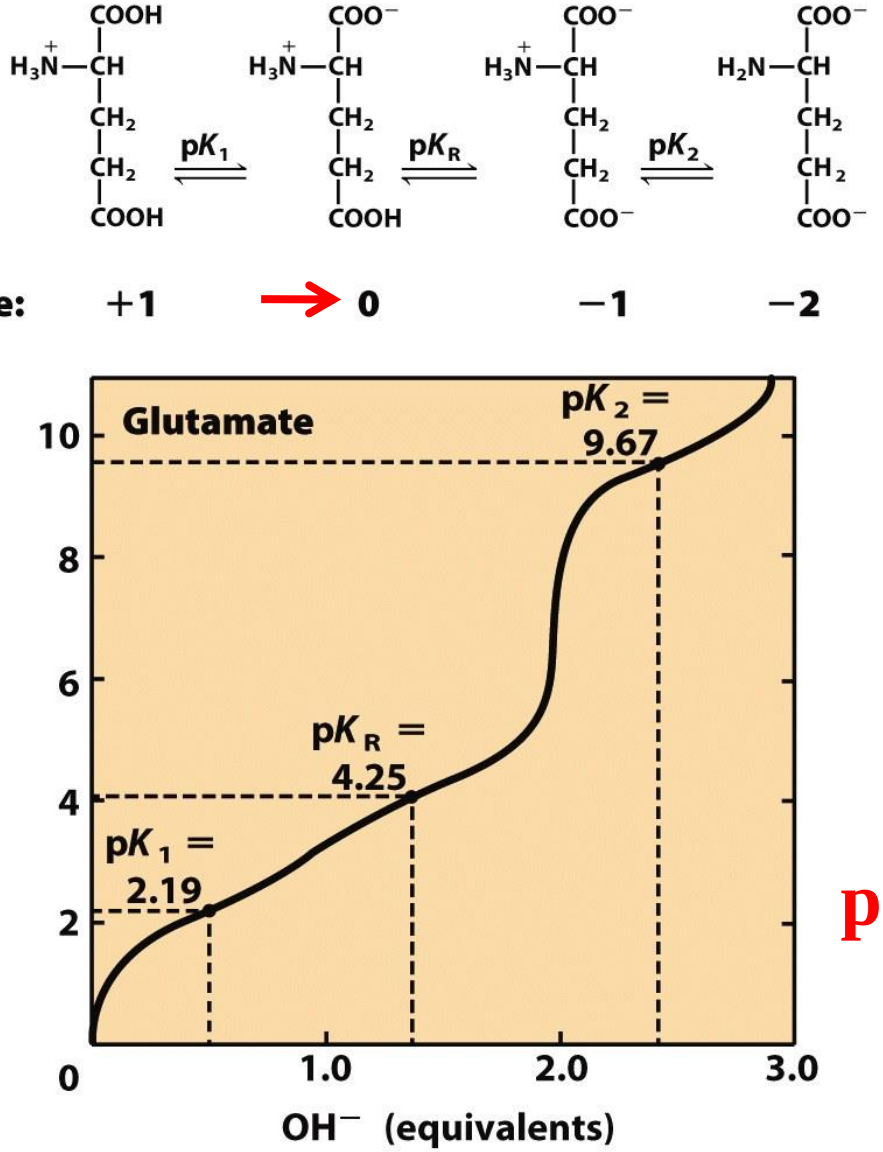
- **Zwitterions predominate at pH values between the p$K_a$ values of the amino and the carboxyl group**
- **For amino acid without ionizable side chains, the Isoelectric Point (pI, Isoelectric pH) is**

$$pI = \frac{pK_1 + pK_2}{2}$$

- **At this point, the net charge is zero**
- **Amino acid is least soluble in water**
- **Amino acid does not migrate in electric field**

# Ionizable side chains can show up in titration curves

- **Ionizable side chains can be also titrated**
- **Titration curves with ionizable side chains are more complex**
- **p$K_a$ values are discernable if two p$K_a$ values are more than two pH units apart**
- **The p$K_a$ of the R group is designated as p$K_R$**




pI = 3.22

Figure 3-12a
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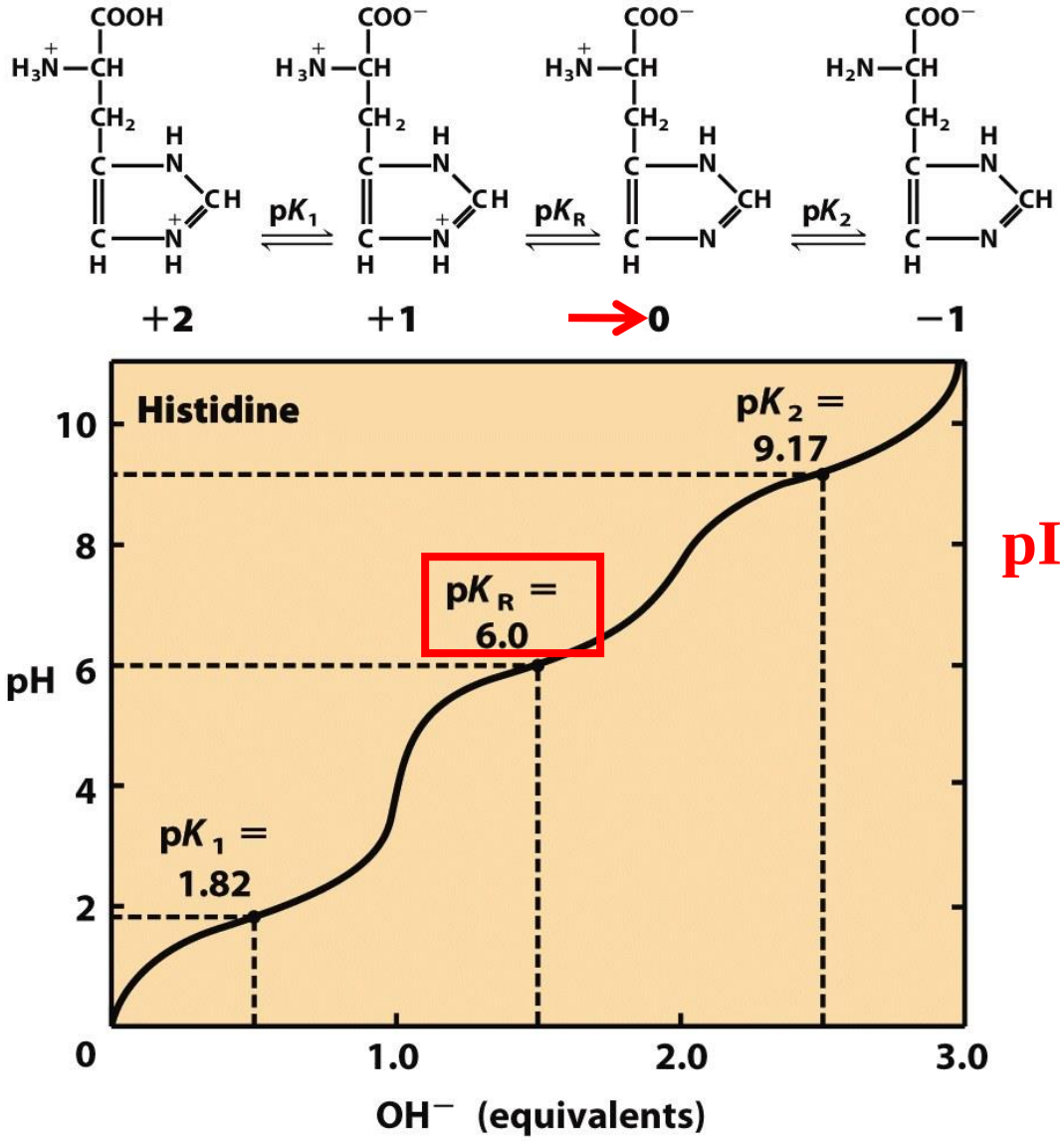
# Titration curve of glutamate




pI = 7.59

Figure 3-12b
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# Titration curve of histidine

# How to calculate the pI when the side-chain is ionizable?

- **Identify species that carries a net zero charge**
- **Identify p$K_a$ value that defines the acid strength of this zwitterion**
- **Identify p$K_a$ value that defines the base strength of this zwitterion**
- **Take the average of these two p$K_a$ values**

# 3. Formation of peptides

- **Peptides: condensation products of amino acids**
- **Peptide bond: linkage between α-carboxyl group of one amino acid to α-amino group of the other amino acid**
- **Polypeptide chain: a series of amino acid residues joined by peptide bonds**
- **Residue: each amino acid unit in a polypeptide**
- **The linking of two amino acids is accompanied by the loss of a molecule of water**
- **Equilibrium of the reaction favors hydrolysis, but peptide bonds are stable kinetically**

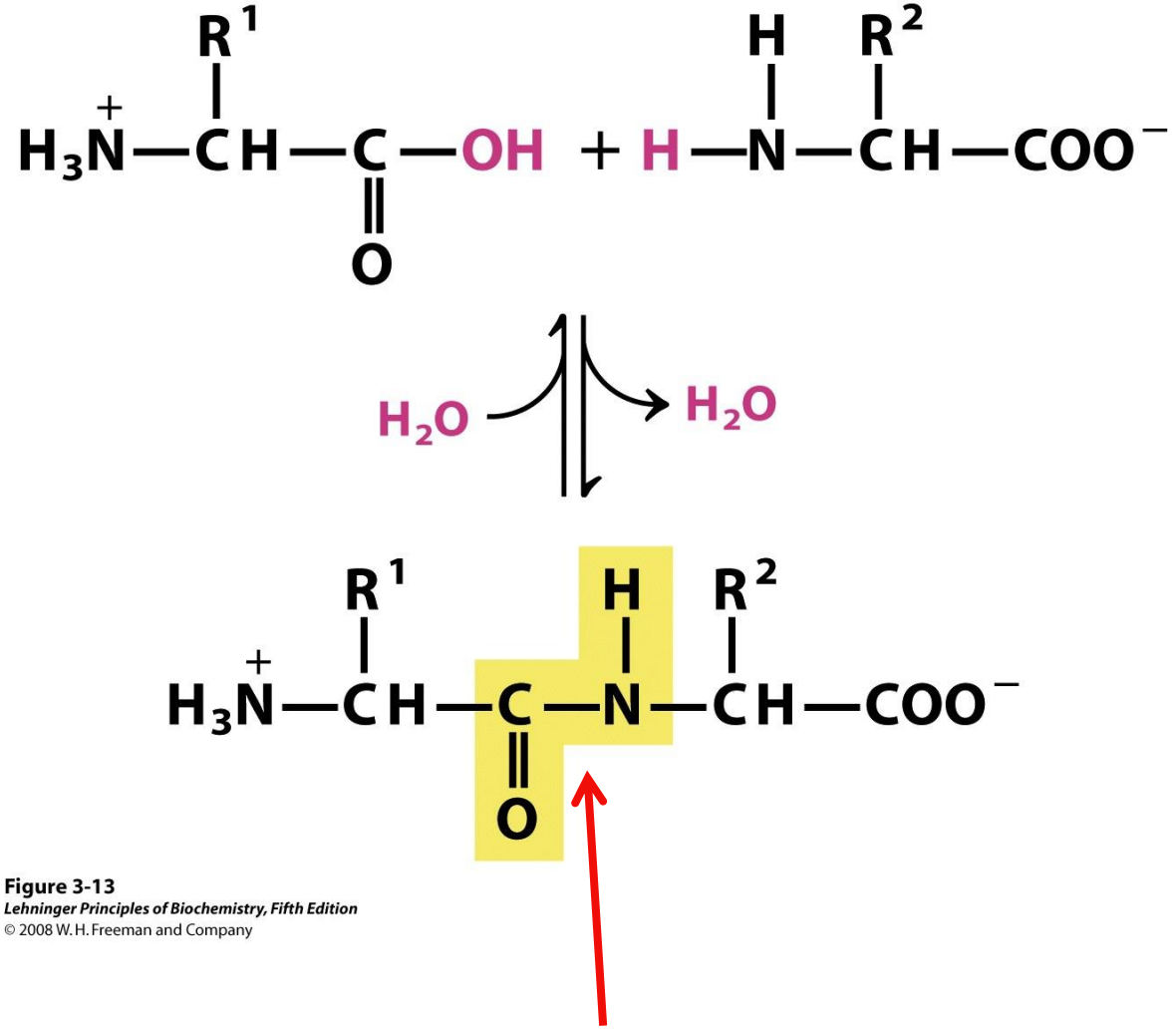


**Figure 3-13**
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# Formation of a peptide bond by condensation

**The formation of a peptide bond is one of the examples of monomers joining to form polymers through condensation reactions.**

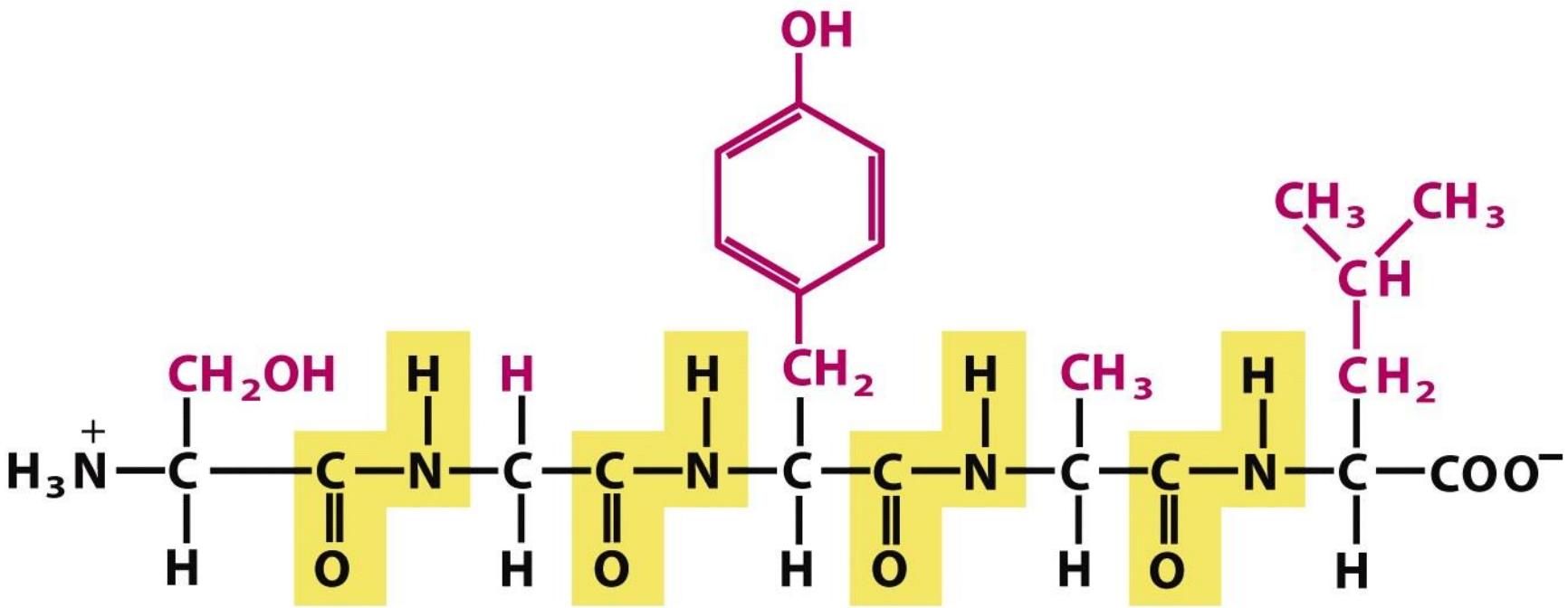



**Figure 3-14**
*Lehninger Principles of Biochemistry, Fifth Edition*


**Peptides are named beginning with the amino-terminal residue, which by convention is placed on the left.**

# The one-letter code and three-letter code

- **Naming starts from the N-terminus**
- **Sequence is written as: Ser-Gly-Tyr-Ala-Leu**
- **Sometimes the one-letter code is used: SGYAL**

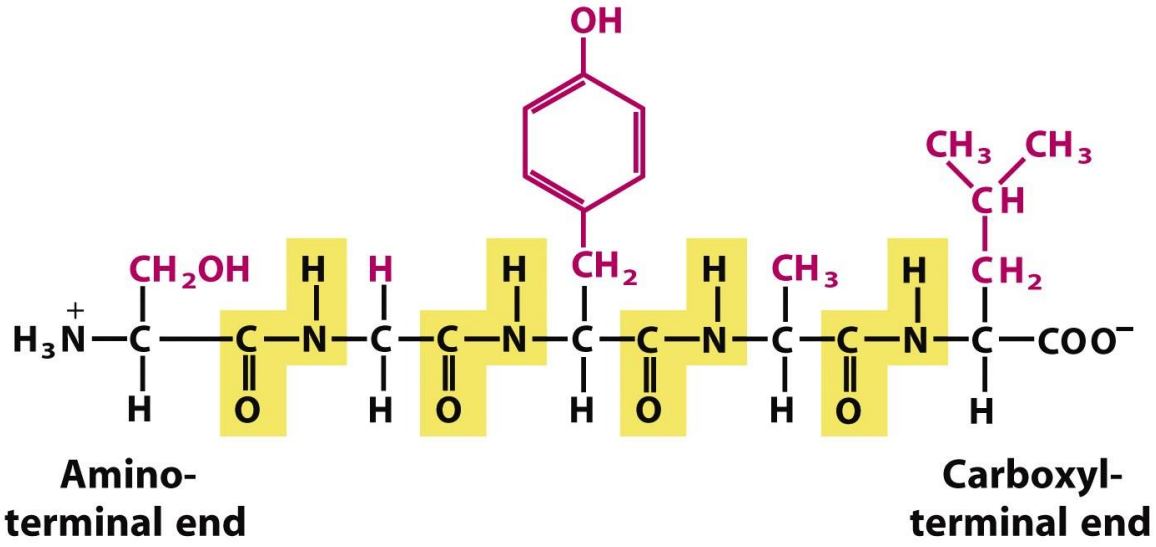
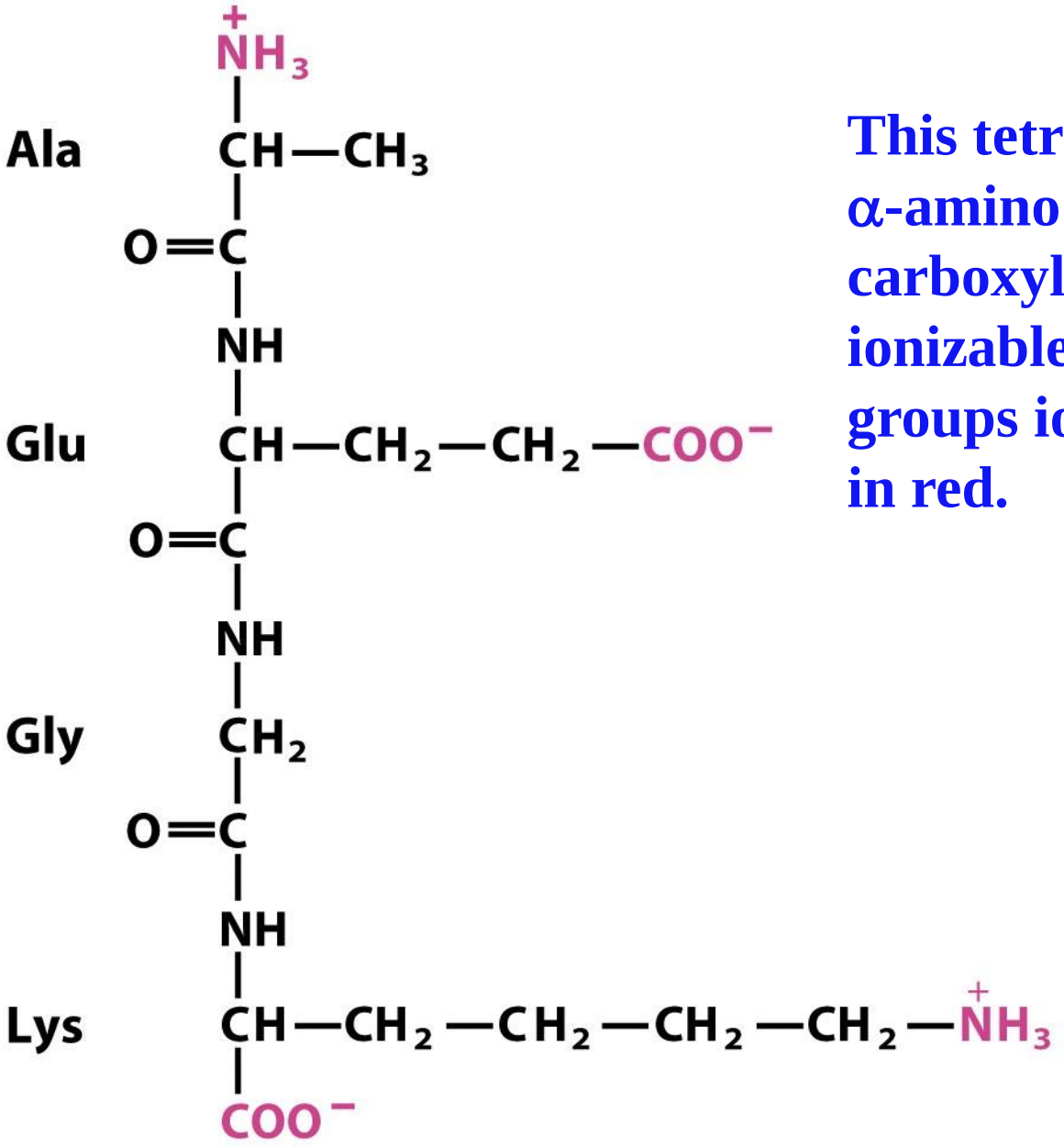



Figure 3-14
*Lehninger Principles of Biochemistry, Fifth Edition*

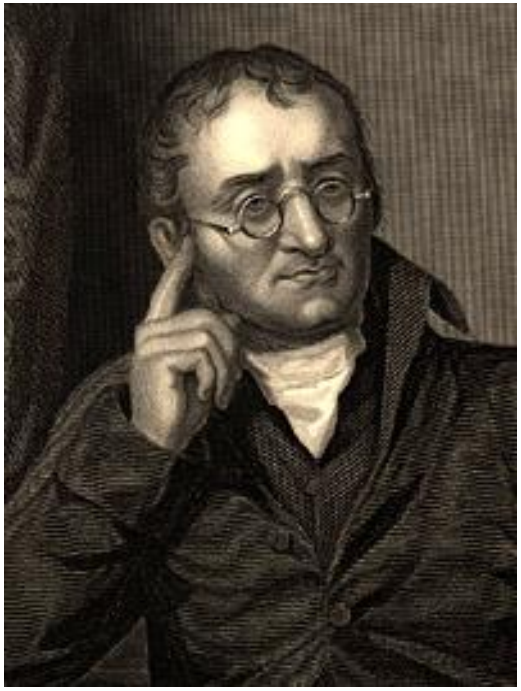



**This tetrapeptide has one free α-amino group, one free α-carboxyl group, and two ionizable R groups. The groups ionized at pH 7.0 are in red.**

**Figure 3-15**
*Lehninger Principles of Biochemistry, Fifth Edition*

# Molecular weight of a protein

- **Dalton (D): a unit of mass nearly equal to that of a hydrogen atom**
- **The mean molecular weight of an amino acid residue in a protein is about 110 dalton, i.e., 0.11 kD**



**John Dalton**

# Peptides: a variety of functions

- **Hormones and pheromones**
  - **insulin**
  - **oxytocin (9 amino acids)**
  - **sex-peptide**
- **Neuropeptides**
  - **substance P (pain mediator)**
- **Antibiotics**
  - **polymyxin B (for Gram - bacteria)**
  - **bacitracin (for Gram + bacteria)**
- **Protection, e.g. toxins**
  - **amanitin (mushrooms)**
  - **conotoxin (cone snails)**
  - **chlorotoxin (scorpions)**

# 4. Proteins are polypeptides

- **Proteins differ in a vast range of sizes, compositions and biological activities**
- **Some proteins contain chemical groups other than amino acids --- conjugated proteins**
- **Cofactor is a general term for functional non-amino acid component --- metal ions or organic molecules**
- **Coenzyme is used to designate an organic cofactors**

  **--- $NAD^+$ in lactate dehydrogenase**
- **Prosthetic groups are covalently attached cofactors**

  **--- Heme in myoglobin**

## TABLE 3–2 Molecular Data on Some Proteins

| | Molecular weight | Number of residues | Number of polypeptide chains |
|---|---|---|---|
| Cytochrome *c* (human) | 13,000 | 104 | 1 |
| Ribonuclease A (bovine pancreas) | 13,700 | 124 | 1 |
| Lysozyme (chicken egg white) | 13,930 | 129 | 1 |
| Myoglobin (equine heart) | 16,890 | 153 | 1 |
| Chymotrypsin (bovine pancreas) | 21,600 | 241 | 3 |
| Chymotrypsinogen (bovine) | 22,000 | 245 | 1 |
| Hemoglobin (human) | 64,500 | 574 | 4 |
| Serum albumin (human) | 68,500 | 609 | 1 |
| Hexokinase (yeast) | 102,000 | 972 | 2 |
| RNA polymerase (*E. coli*) | 450,000 | 4,158 | 5 |
| Apolipoprotein B (human) | 513,000 | 4,536 | 1 |
| Glutamine synthetase (*E. coli*) | 619,000 | 5,628 | 12 |
| Titin (human) | 2,993,000 | 26,926 | 1 |

**Table 3-2**
***Lehninger Principles of Biochemistry, Fifth Edition***

## TABLE 3–3 Amino Acid Composition of Two Proteins

| Amino acid | Number of residues per molecule of protein* | |
|---|---|---|
| | Bovine cytochrome c | Bovine chymotrypsinogen |
| Ala | 6 | 22 |
| Arg | 2 | 4 |
| Asn | 5 | 15 |
| Asp | 3 | 8 |
| Cys | 2 | 10 |
| Gln | 3 | 10 |
| Glu | 9 | 5 |
| Gly | 14 | 23 |
| His | 3 | 2 |
| Ile | 6 | 10 |
| Leu | 6 | 19 |
| Lys | 18 | 14 |
| Met | 2 | 2 |
| Phe | 4 | 6 |
| Pro | 4 | 9 |
| Ser | 1 | 28 |
| Thr | 8 | 23 |
| Trp | 1 | 8 |
| Tyr | 4 | 4 |
| Val | 3 | 23 |
| Total | 104 | 245 |

*In some common analyses, such as acid hydrolysis, Asp and Asn are not readily distinguished from each other and are together designated Asx (or B). Similarly, when Glu and Gln cannot be distinguished, they are together designated Glx (or Z). In addition, Trp is destroyed by acid hydrolysis. Additional procedures must be employed to obtain an accurate assessment of complete amino acid content.

**Table 3-3**

***Lehninger Principles of Biochemistry, Fifth Edition***

## TABLE 3–4 Conjugated Proteins

| Class | Prosthetic group | Example |
|---|---|---|
| Lipoproteins | Lipids | $\beta_1$-Lipoprotein of blood |
| Glycoproteins | Carbohydrates | Immunoglobulin G |
| Phosphoproteins | Phosphate groups | Casein of milk |
| Hemoproteins | Heme (iron porphyrin) | Hemoglobin |
| Flavoproteins | Flavin nucleotides | Succinate dehydrogenase |
| Metalloproteins | Iron | Ferritin |
| | Zinc | Alcohol dehydrogenase |
| | Calcium | Calmodulin |
| | Molybdenum | Dinitrogenase |
| | Copper | Plastocyanin |

**Table 3-4**
***Lehninger Principles of Biochemistry, Fifth Edition***

# What to learn about a protein?

- **What is its sequence and composition?**
- **What is its three-dimensional structure?**
- **How does it find its native fold?**
- **How does it achieve its biochemical role?**
- **How is its function regulated?**
- **How does it interacts with other macromolecules?**
- **How is it related to other proteins?**
- **Where is it localized within the cell?**

# 5. Working with proteins

**Major steps in purifying an intracellular protein:**

1. **Disrupt cell membrane to obtain homogenate --- crude extract**
2. **Fractionate the mixture by centrifugation**
3. **Collect each fraction for assay**
4. **Apply further techniques to enrich the protein**

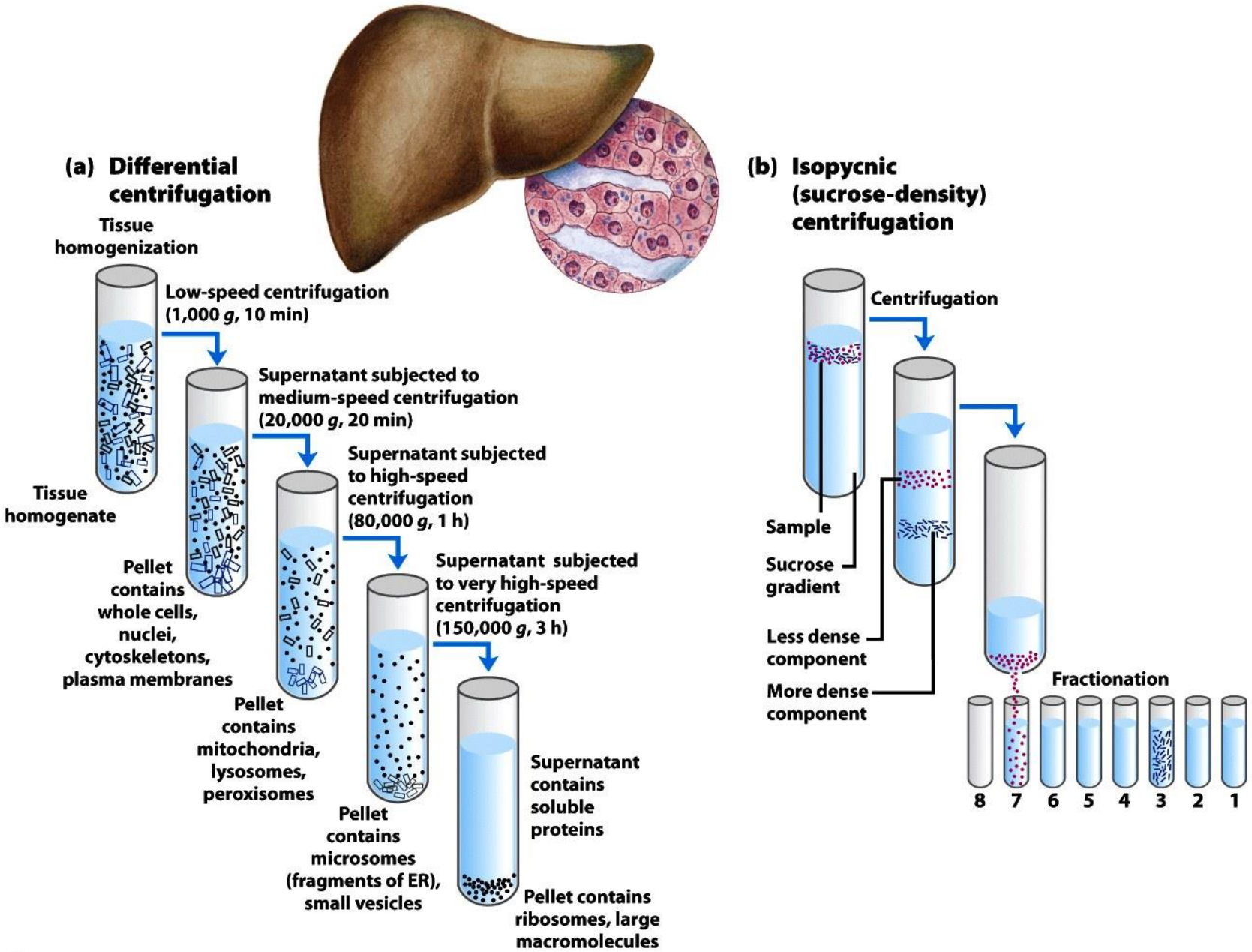



**Figure 1-8**
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# Subcellular fractionation of tissue

# A mixture of proteins can be separated

- **Separation relies on differences in physicochemical properties**
  - **Charge**
  - **Size**
  - **Affinity for a ligand**
  - **Solubility**
  - **Hydrophobicity**
  - **Thermal stability**
- **Chromatography is commonly used for preparative separation**

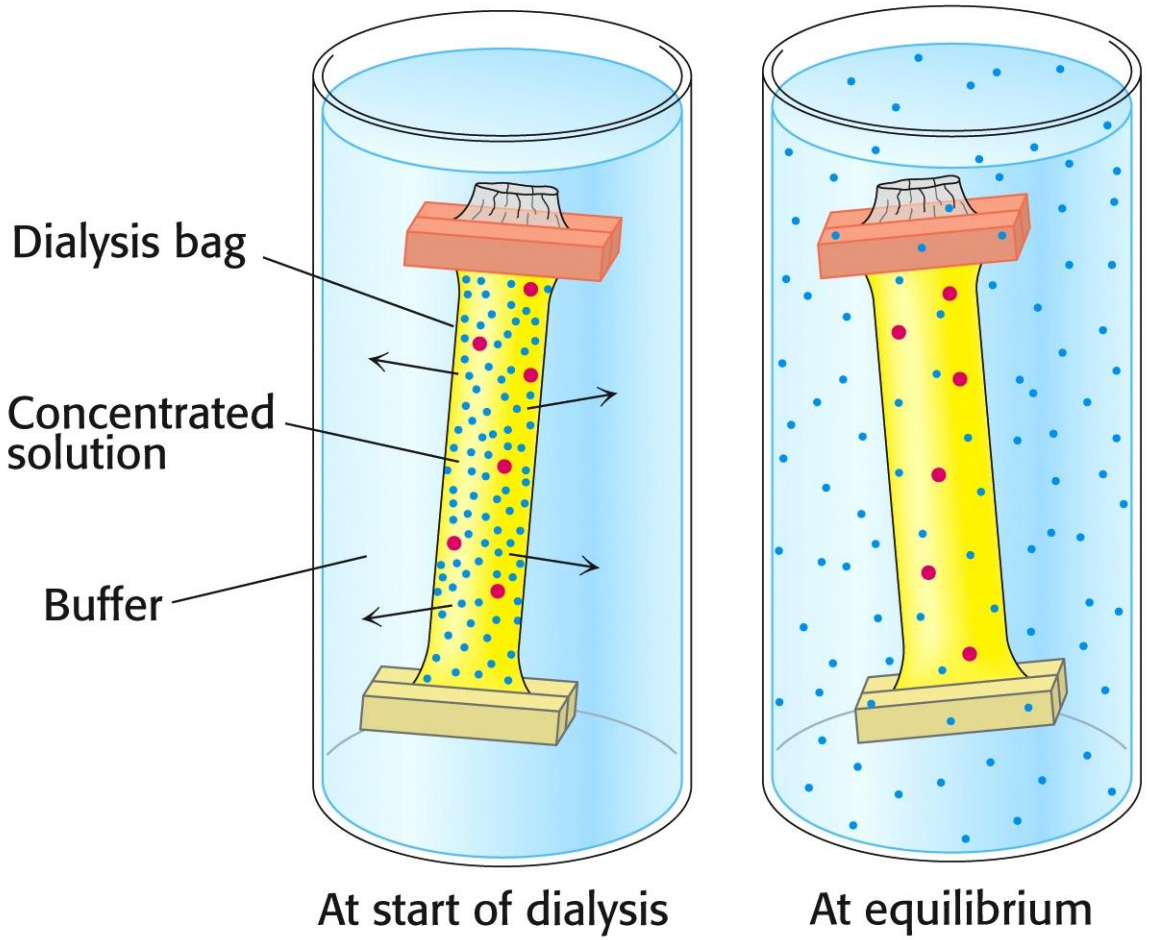
# Techniques used in protein purification

- **Salting out**
- **Dialysis**
- **Chromatography**
  - **Size-exclusion chromatography**
  - **Ion-exchange chromatography**
  - **Affinity chromatography**
  - **High-performance liquid chromatography**

# Salting-out

- **The solubility of proteins is generally lowered at high salt concentration**
- **The addition of certain salts in the right amounts can selectively precipitate some proteins, while others remain in solution**
- **Ammonium sulfate $(NH_4)_2SO_4$ is commonly chosen as the salt for 'salting-out': high concentration can be achieved; does not usually denature proteins; it's reversible; not expensive.**

# Dialysis




**Protein molecules (red) are retained within the dialysis bag, whereas small molecules (blue) diffuse into the medium.**

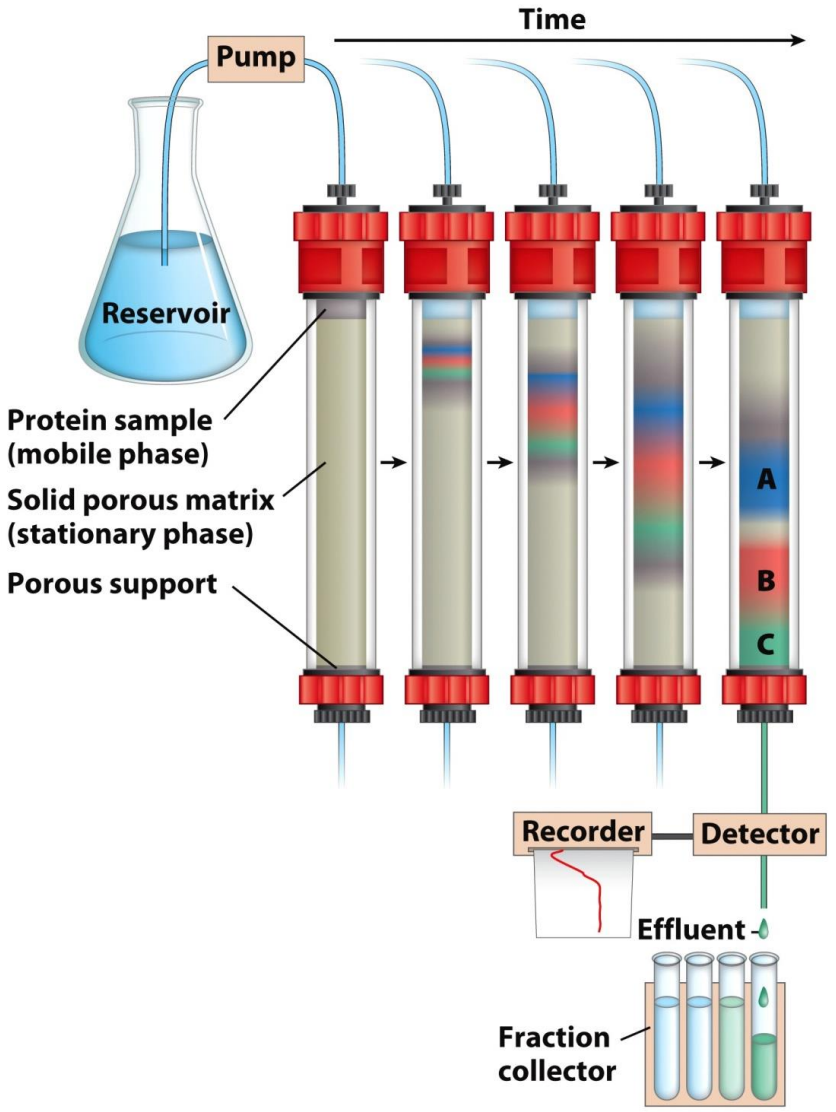
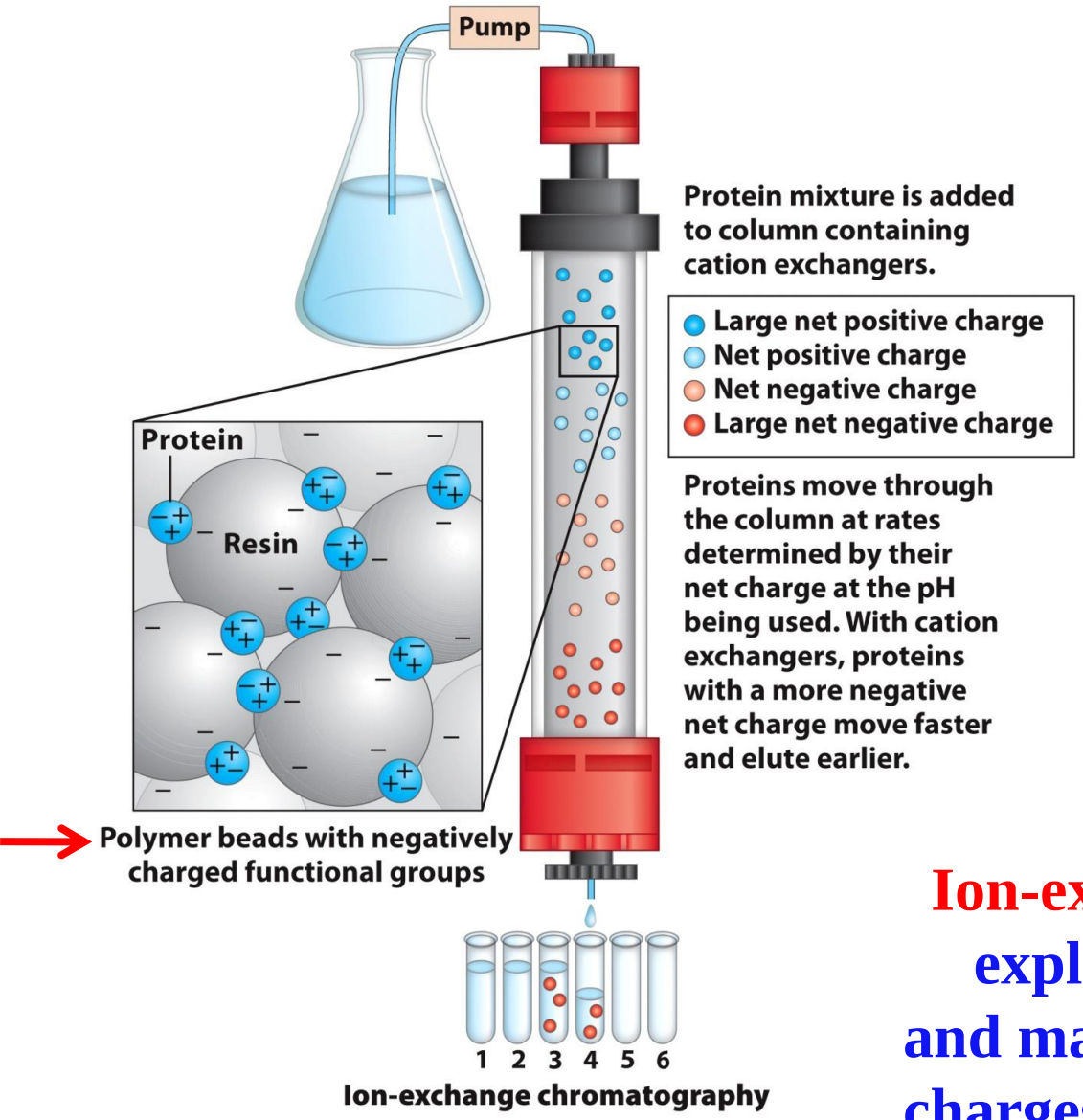



**Figure 3-16**
*Lehninger Principles of Biochemistry*, Sixth Edition


**Column chromatography allows separation of a mixture of proteins over a solid phase (porous matrix) using a liquid phase to mobilize the proteins.**

# Column chromatography

# Separation by charge

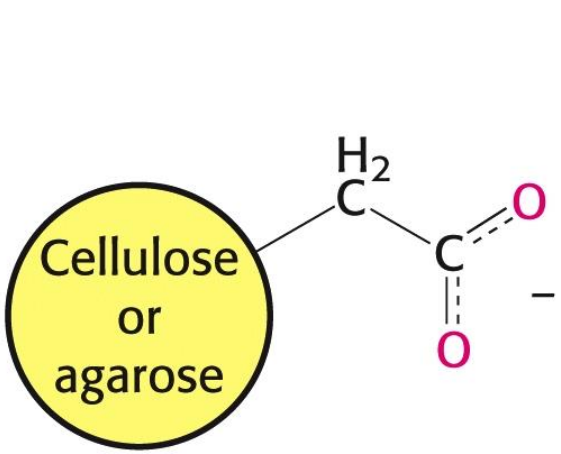



**Ion-exchange chromatography exploits differences the sign and magnitude of the net electric charges of proteins at a given pH**

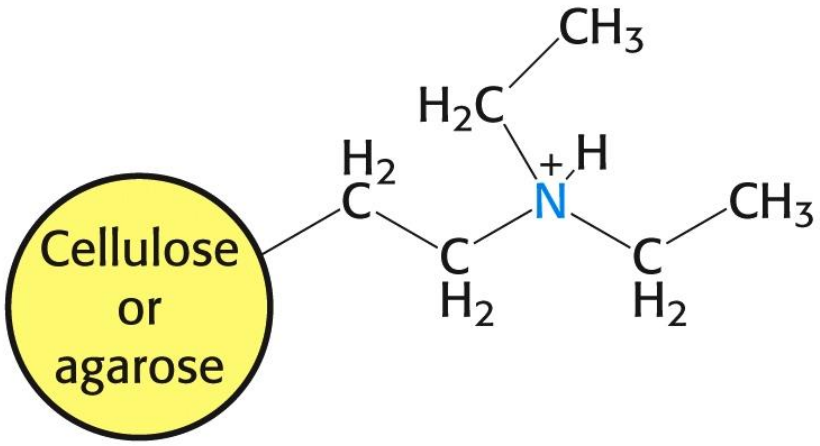
**Figure 3-17a**
*Lehninger Principles of Biochemistry*, Sixth Edition

# Ion-exchange chromatography matrix

**CM-cellulose: negatively charged**
**DEAE-cellulose: positively charged**




**Carboxymethyl (CM) group**
(ionized form)




**Diethylaminoethyl (DEAE) group**
(protonated form)

# Separation by size

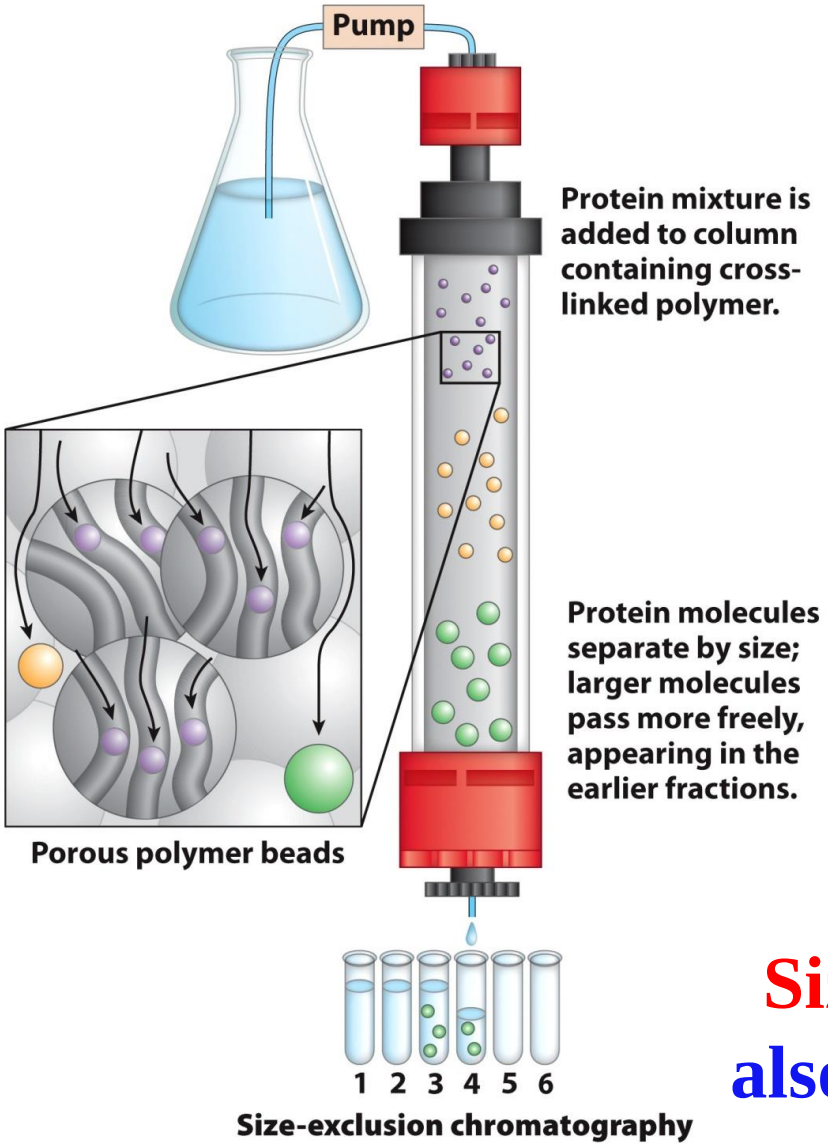



**Figure 3-17b**
*Lehninger Principles of Biochemistry*, Sixth Edition


**Size-exclusion chromatography, also called gel filtration, separates proteins according to size.**

# Separation by affinity

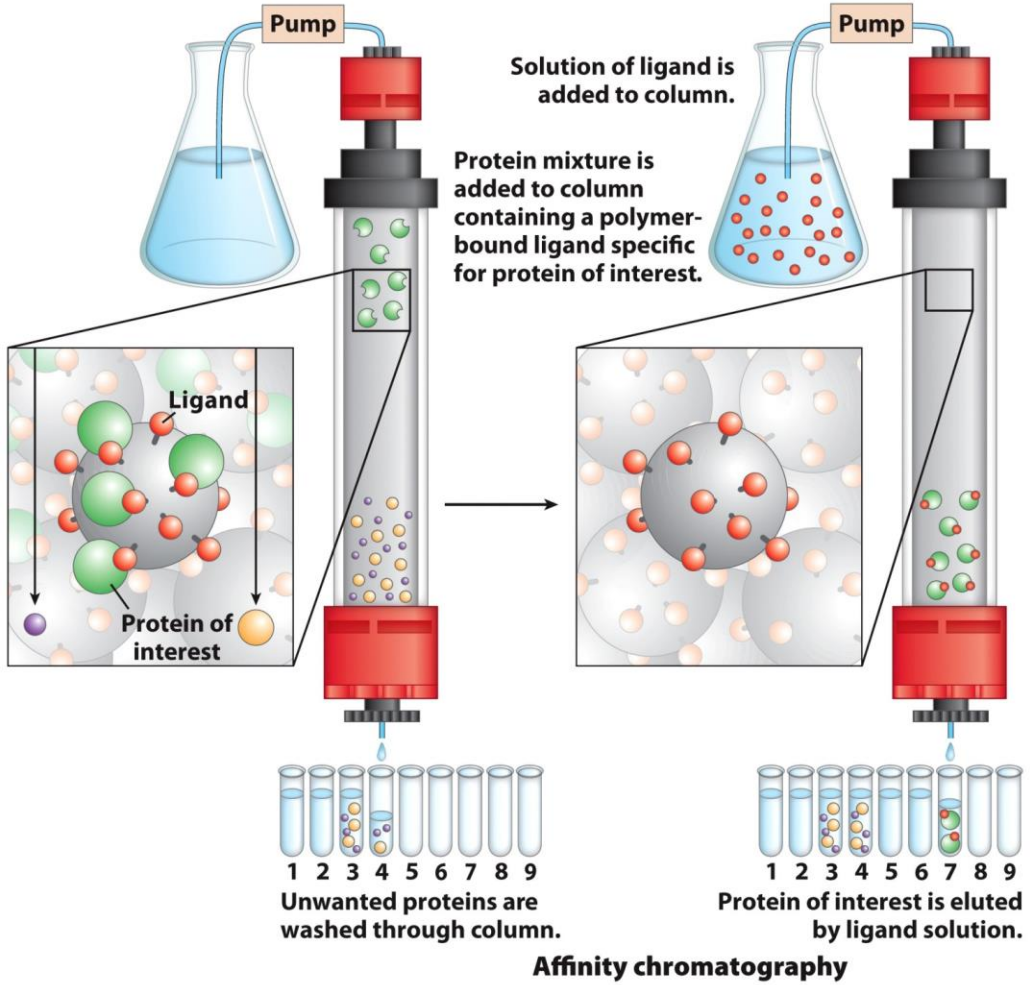



**Figure 3-17c**
*Lehninger Principles of Biochemistry*, Sixth Edition


**Affinity chromatography separates proteins by their binding specificities**

# High-performance liquid chromatography (HPLC)

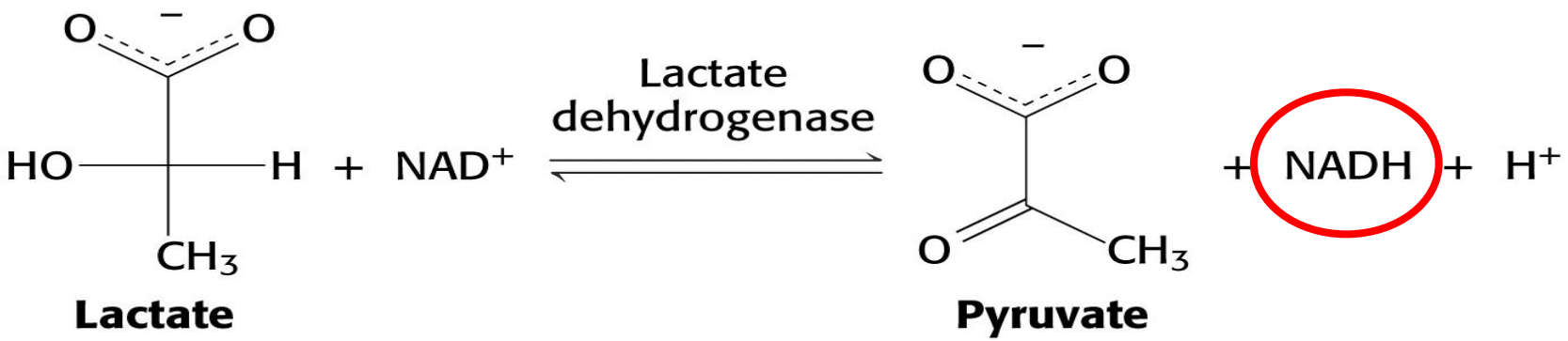
- **HPLC makes use of high-pressure pumps that speed up the movement of protein molecules down the column**
- **The column is made of higher-quality, greater-resolving power chromatographic materials that can withstand the high pressure**

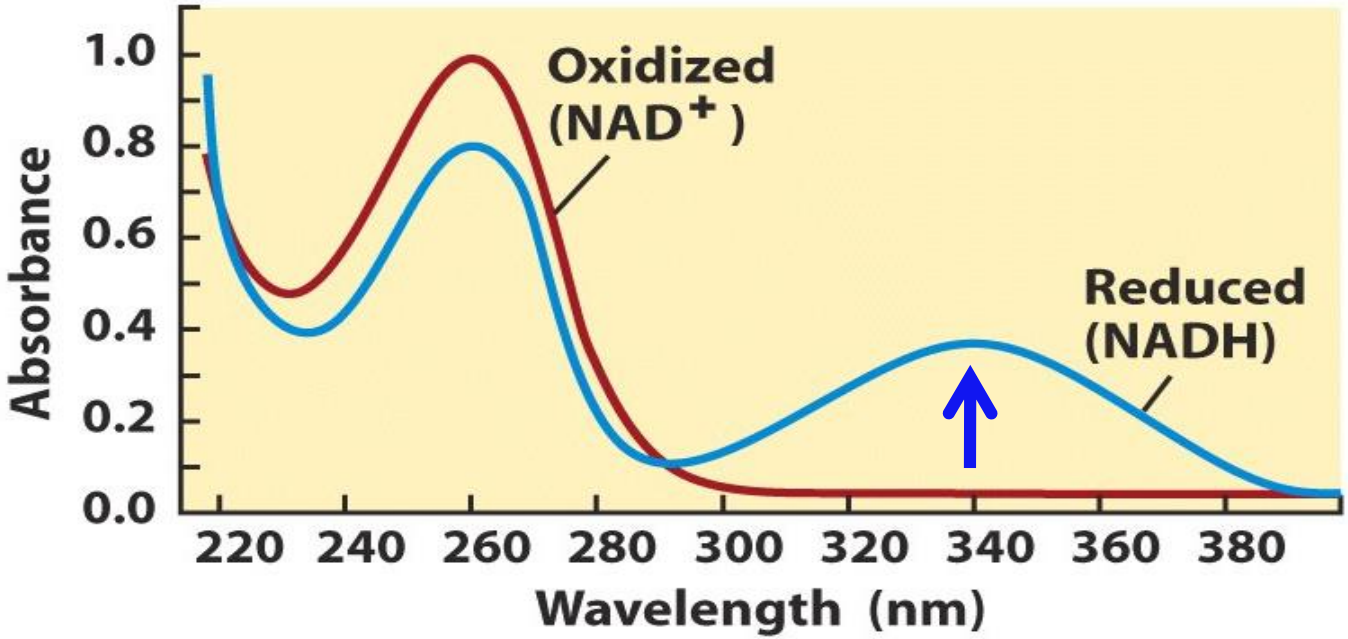
# Major methods in purifying a protein*****

| Method | Principle |
| --- | --- |
| Salting out | Solubility |
| Dialysis | Size |
| Size-exclusion chromatography | Size |
| Ion-exchange chromatography | Charge |
| Affinity chromatography | Binding affinity |

# How to detect and quantify the protein being purified?

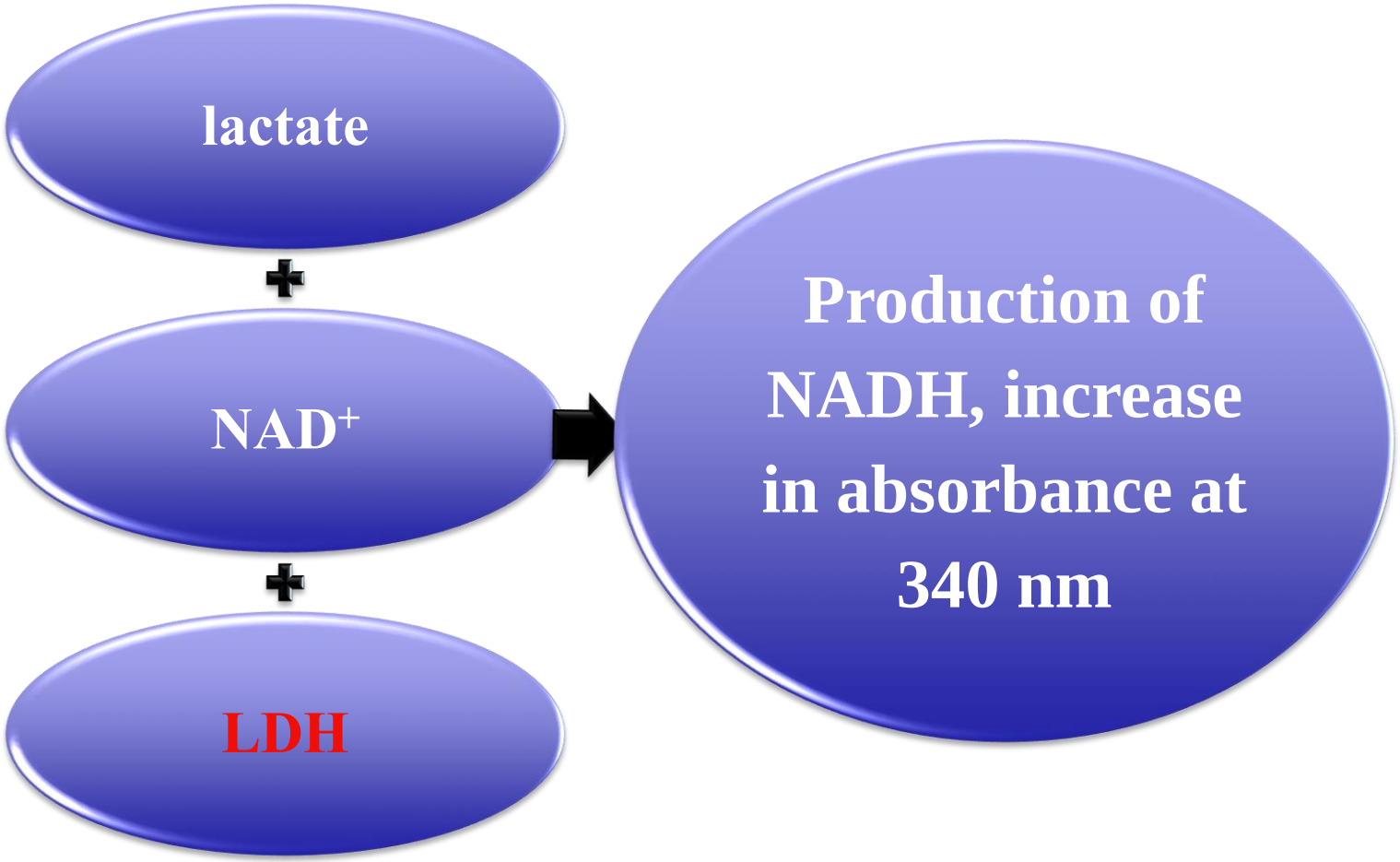
- **An assay needs to be designed based on the unique properties of the protein**
- **The more specific the assay, the more effective the purification**
- **The key point of purification is to maximize the specific activity**




**The production of NADH during an enzyme-catalyzed reaction can be conveniently followed by observing the appearance of the absorbance at 340 nm.**

# Design an assay for lactate dehydrogenase



# Specific activity describes the purity of the protein of interest

- **Proteins in a complex mixture often require more than one purification to completely isolate the protein of interest.**
- **During purification, determination of the location of the protein of interest can be performed by tracking its behavior.**
- **If a protein has a specific function (e.g., binding insulin), the fraction that binds insulin best after each purification step will contain the most of the protein of interest.**
- **The function of the protein is called the “activity.”**
- **The ratio of activity to total protein concentration is called the “specific activity.”**

# Activity versus specific activity

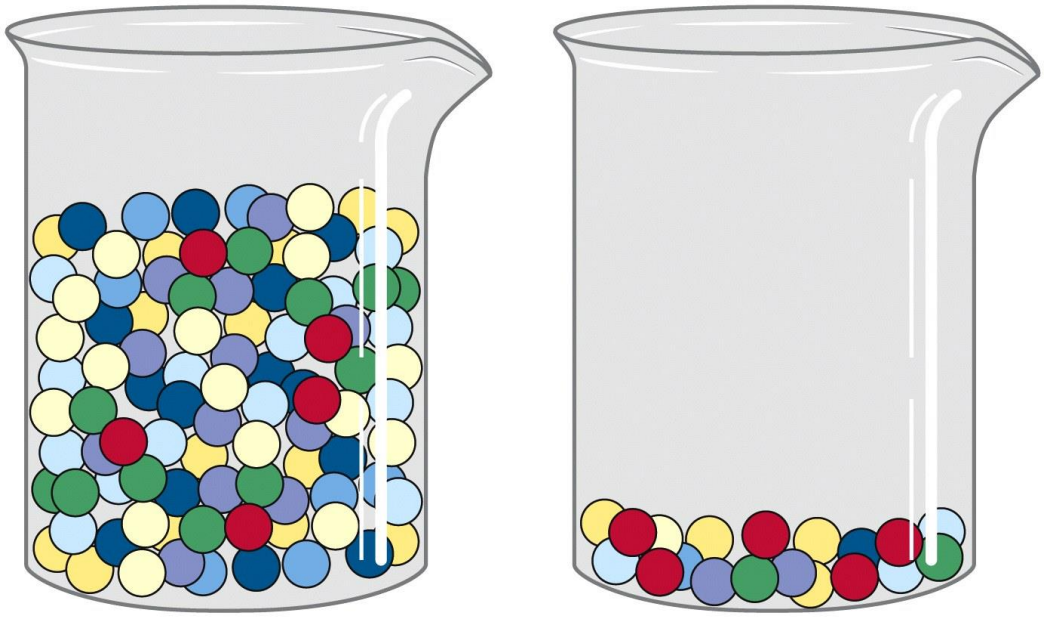


**Figure 3-22**
***Lehninger Principles of Biochemistry, Fifth Edition***


**Activity: the total units of enzyme in a solution**

**Specific activity = enzyme activity/amount of protein**

# Spectroscopic detection of proteins

- **The aromatic amino acids absorb light in the UV region**
- **Proteins typically have UV absorbance maxima around 275-280 nm**
- **Tryptophan and tyrosine are the strongest chromophores**
- **Concentration can be determined by UV-visible spectrophotometry**

**TABLE 3–5 A Purification Table for a Hypothetical Enzyme**

| Procedure or step | Fraction volume (mL) | Total protein (mg) | Activity (units) | Specific activity (units/mg) |
|---|---|---|---|---|
| 1. Crude cellular extract | 1,400 | 10,000 | 100,000 | 10 |
| 2. Precipitation with ammonium sulfate | 280 | 3,000 | 96,000 | 32 |
| 3. Ion-exchange chromatography | 90 | 400 | 80,000 | 200 |
| 4. Size-exclusion chromatography | 80 | 100 | 60,000 | 600 |
| 5. Affinity chromatography | 6 | 3 | 45,000 | 15,000 |

**Note:** All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 91.

Table 3-5

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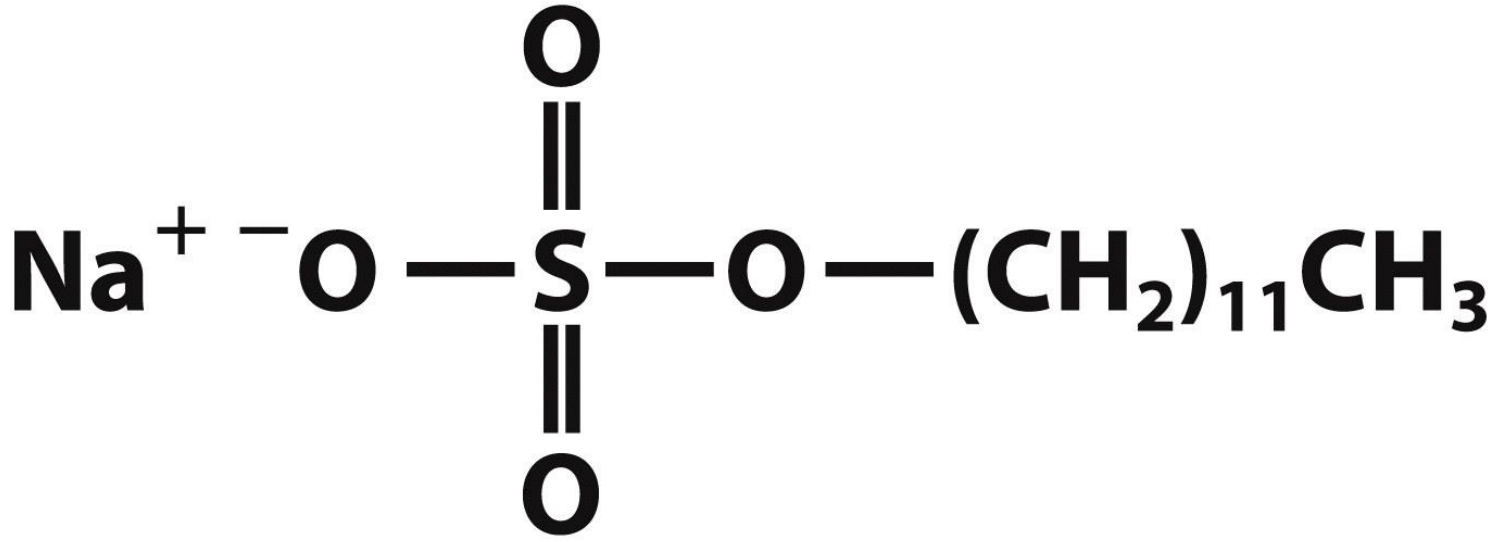
# Electrophoresis for protein separation and analysis

- **Separation in analytical scale is commonly done by electrophoresis**
  - **Electric field pulls proteins according to their charge**
  - **Gel matrix hinders mobility of proteins according to their size and shape**
- **Proteins can be visualized as well as separated by electrophoresis**
- **Used to determine pI and MW**

# SDS-PAGE

## (SDS-polyacrylamide gel electrophoresis )

- **SDS binds to most proteins in amounts roughly proportional to the MW of the protein ( 1 SDS: 2 amino acids)**
- **SDS disrupts nearly all noncovalent interactions in native proteins and unfold the proteins**
- **SDS gives all proteins an uniformly negative charge (similar charge-to-mass ratio)**
  - **The native shape of proteins does not matter**
  - **Rate of movement will only depend on size: small proteins will move faster**




# Sodium dodecyl sulfate (SDS)

**Unnumbered 3 p89**
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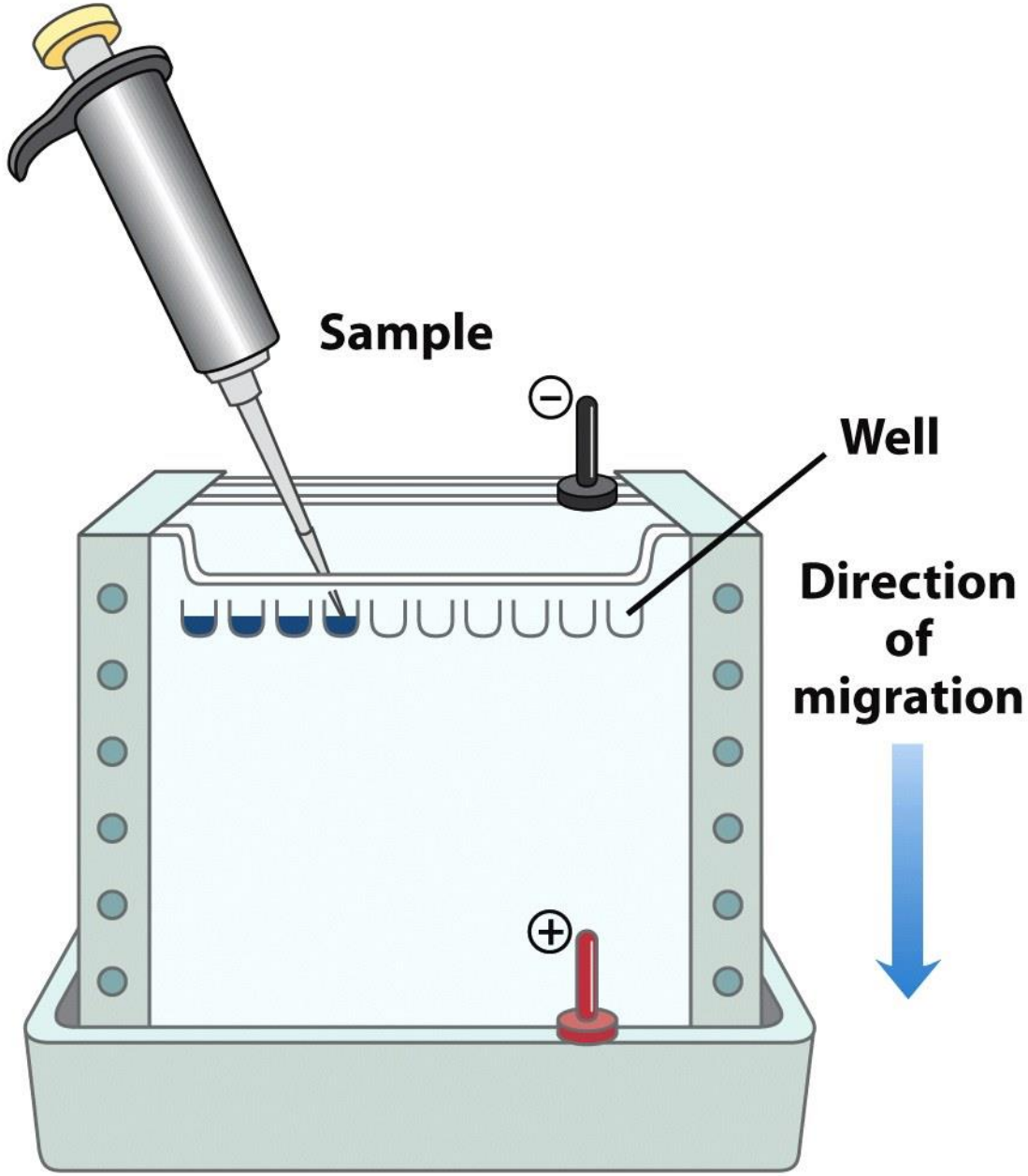


**Figure 3-18a**
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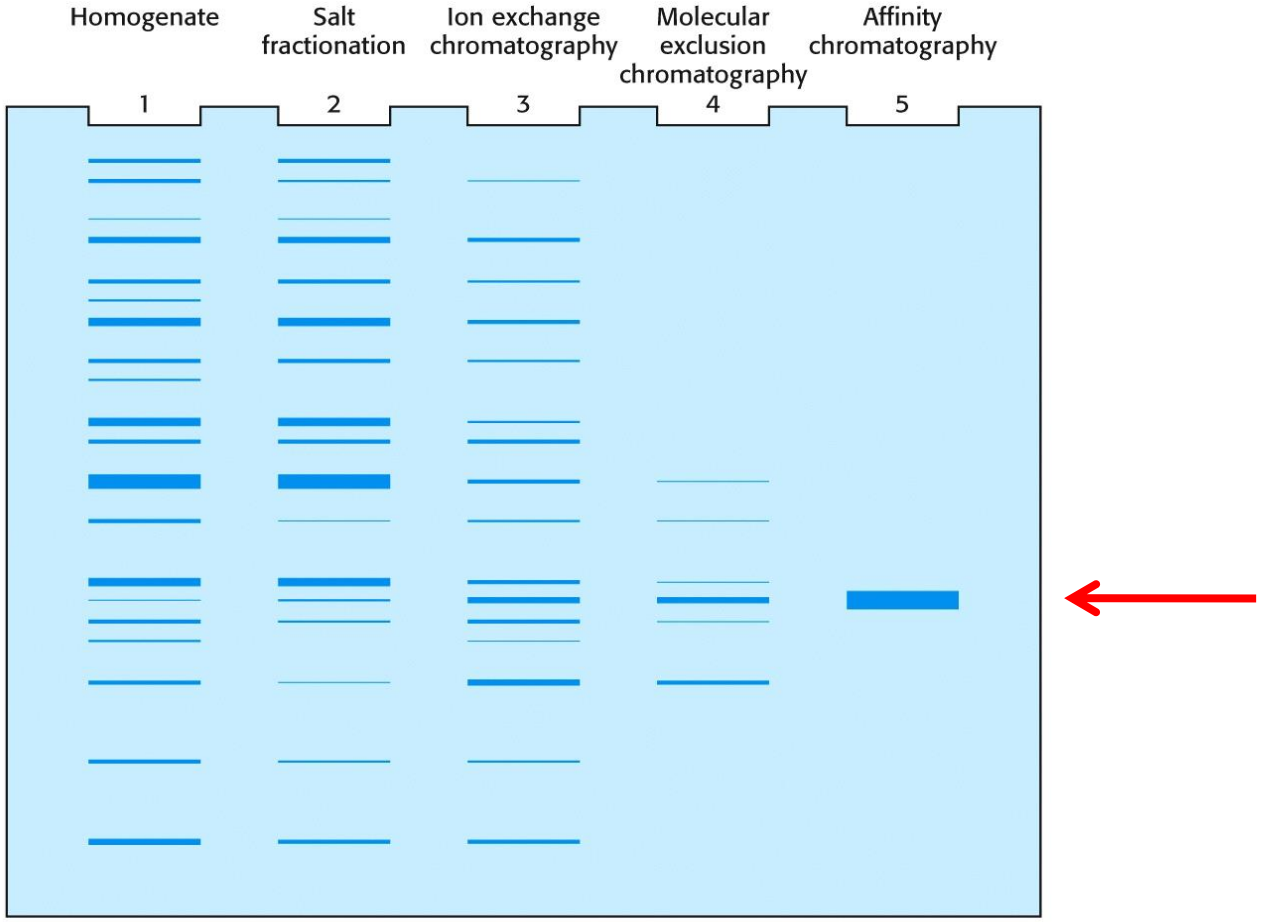
# Electrophoresis




**The purification scheme was analyzed by SDS-PAGE. Each lane contained 50 µg of sample. The effectiveness of the purification can be seen as the band for the protein of interest becomes more prominent relative to other bands.**

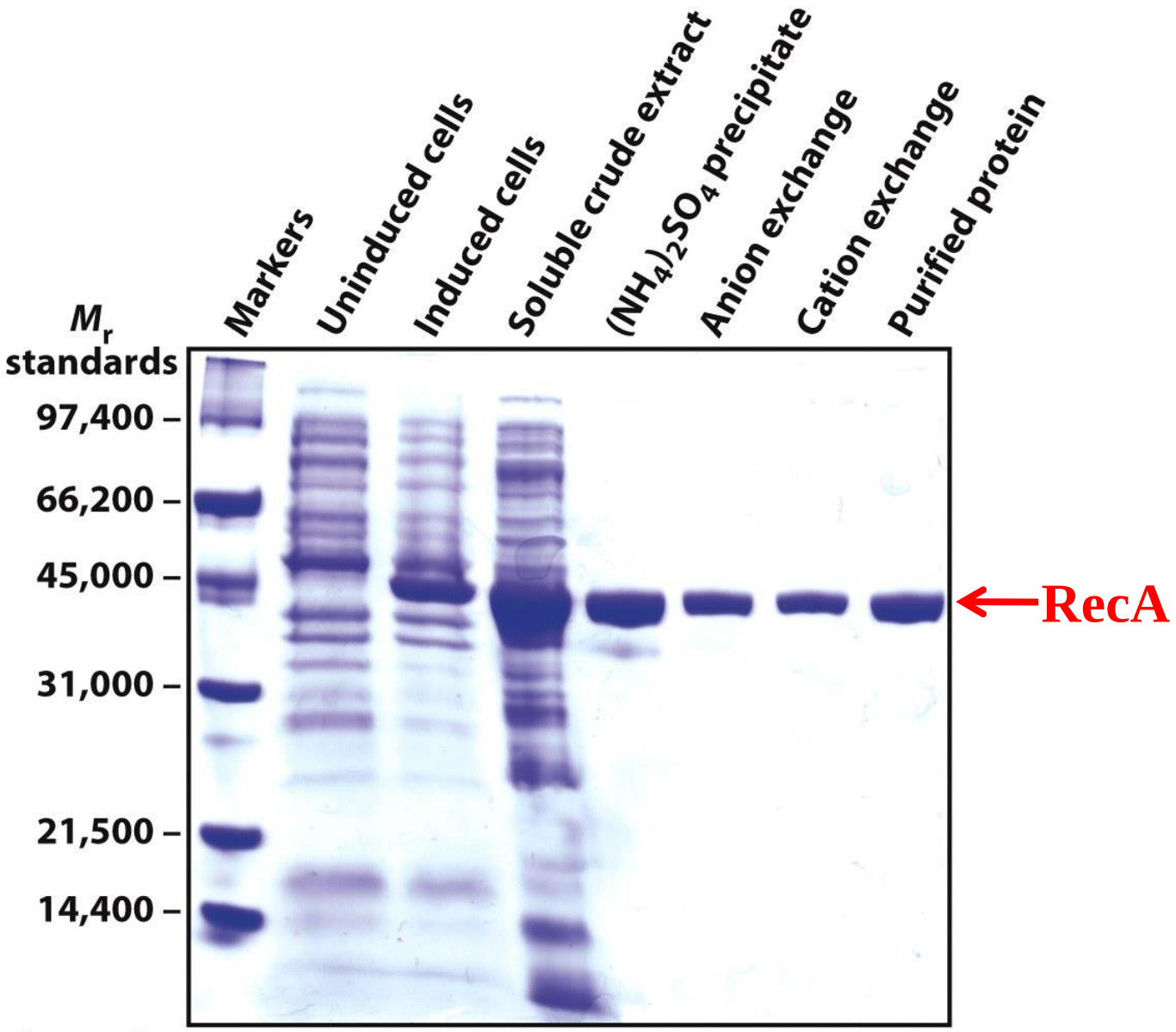



**Figure 3-18b**
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# Purification of the RecA protein of *Escherichia coli*

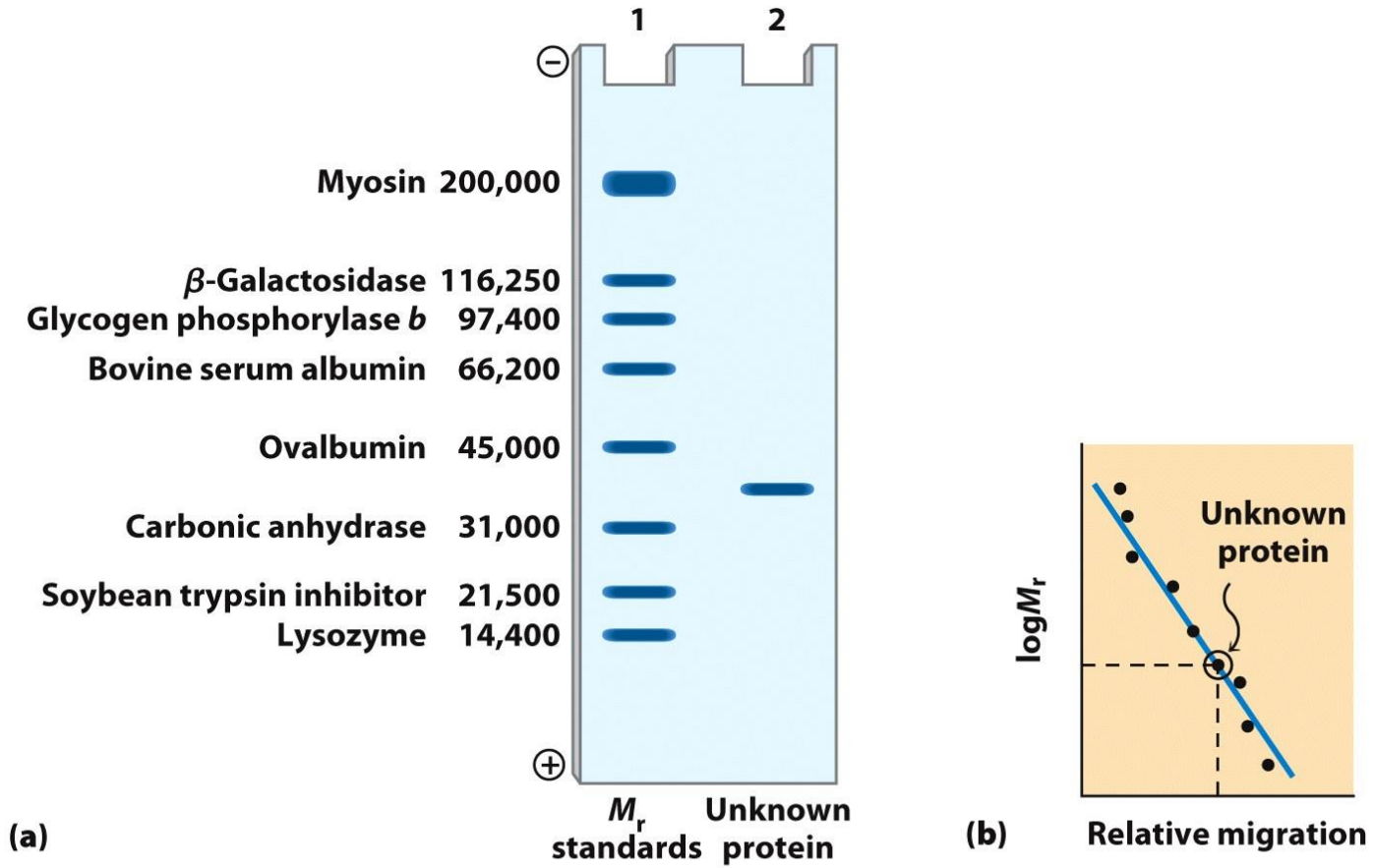


**Figure 3-19**
***Lehninger Principles of Biochemistry, Fifth Edition***


# Estimating the molecular weight of a protein

## TABLE 3–6 The Isoelectric Points of Some Proteins

| Protein | pI |
|---|---|
| Pepsin | <1.0 |
| Egg albumin | 4.6 |
| Serum albumin | 4.9 |
| Urease | 5.0 |
| β-Lactoglobulin | 5.2 |
| Hemoglobin | 6.8 |
| Myoglobin | 7.0 |
| Chymotrypsinogen | 9.5 |
| Cytochrome *c* | 10.7 |
| Lysozyme | 11.0 |

**Table 3-6**
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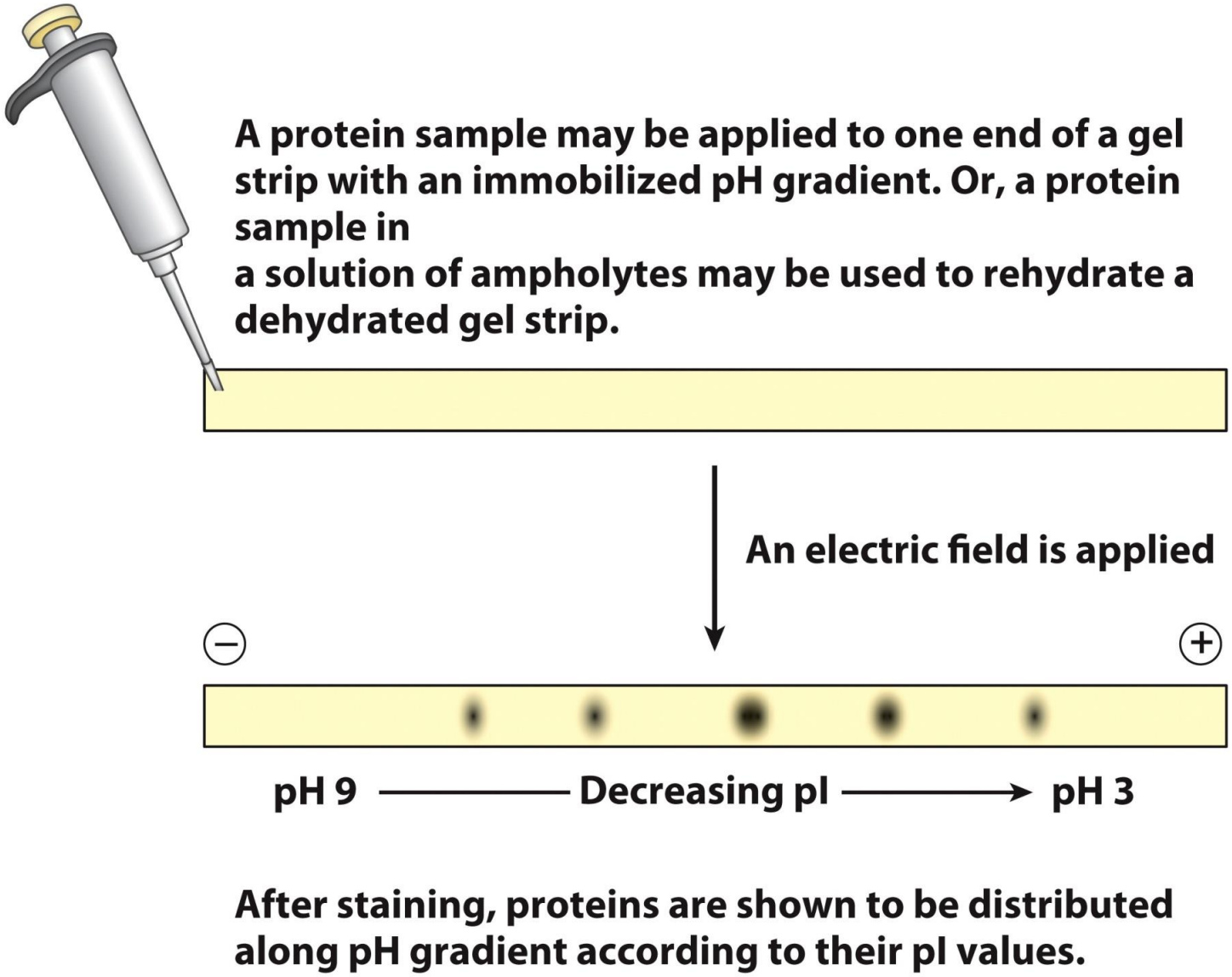


**Figure 3-20**
*Lehninger Principles of Biochemistry*, Sixth Edition


**Isoelectric focusing separates proteins according to their isoelectric points (pI)**

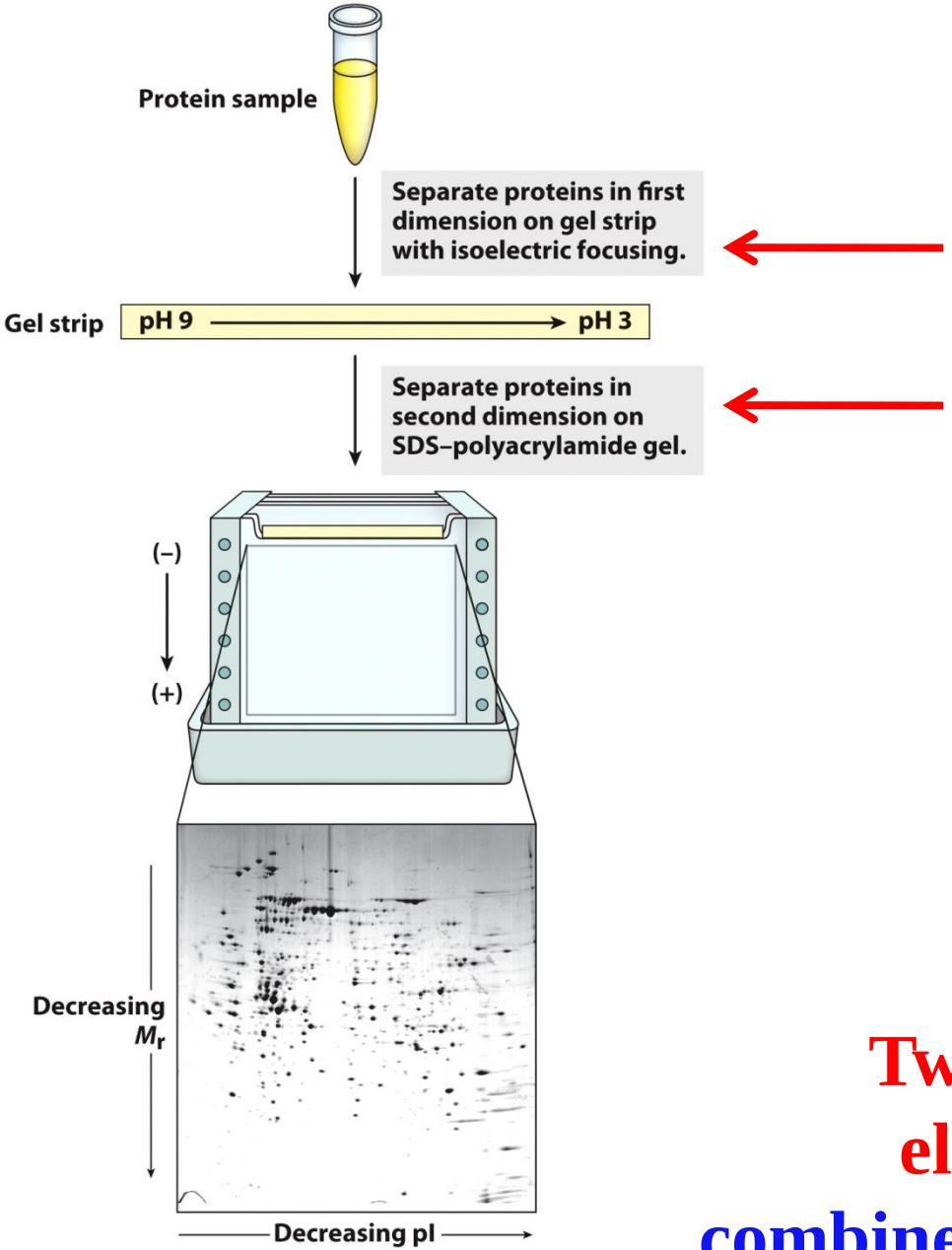


**Figure 3-21**
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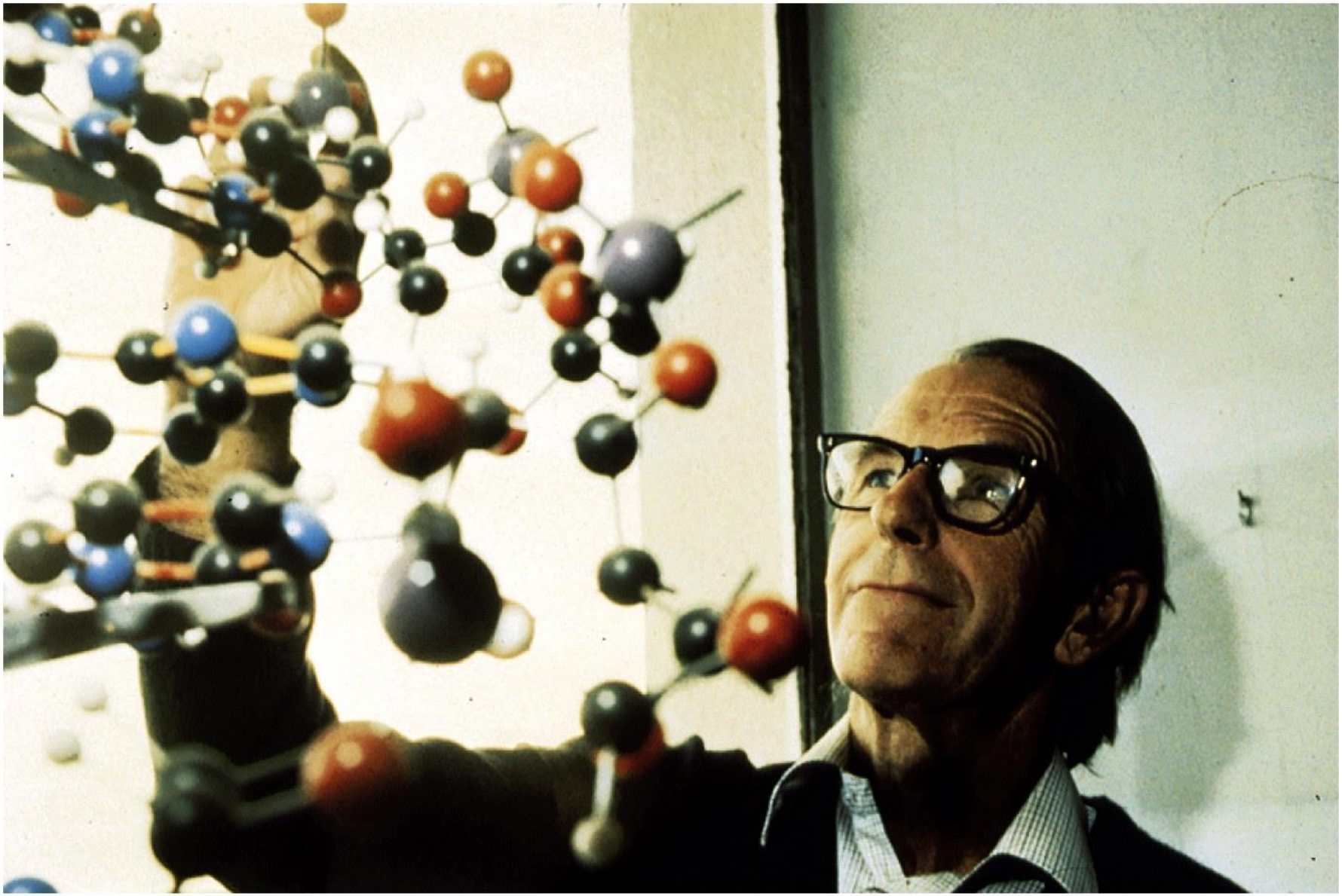
# Two-dimensional electrophoresis: combine isoelectric focusing and electrophoresis



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# Frederick Sanger (developed FDNB)

# Steps in sequencing a polypeptide

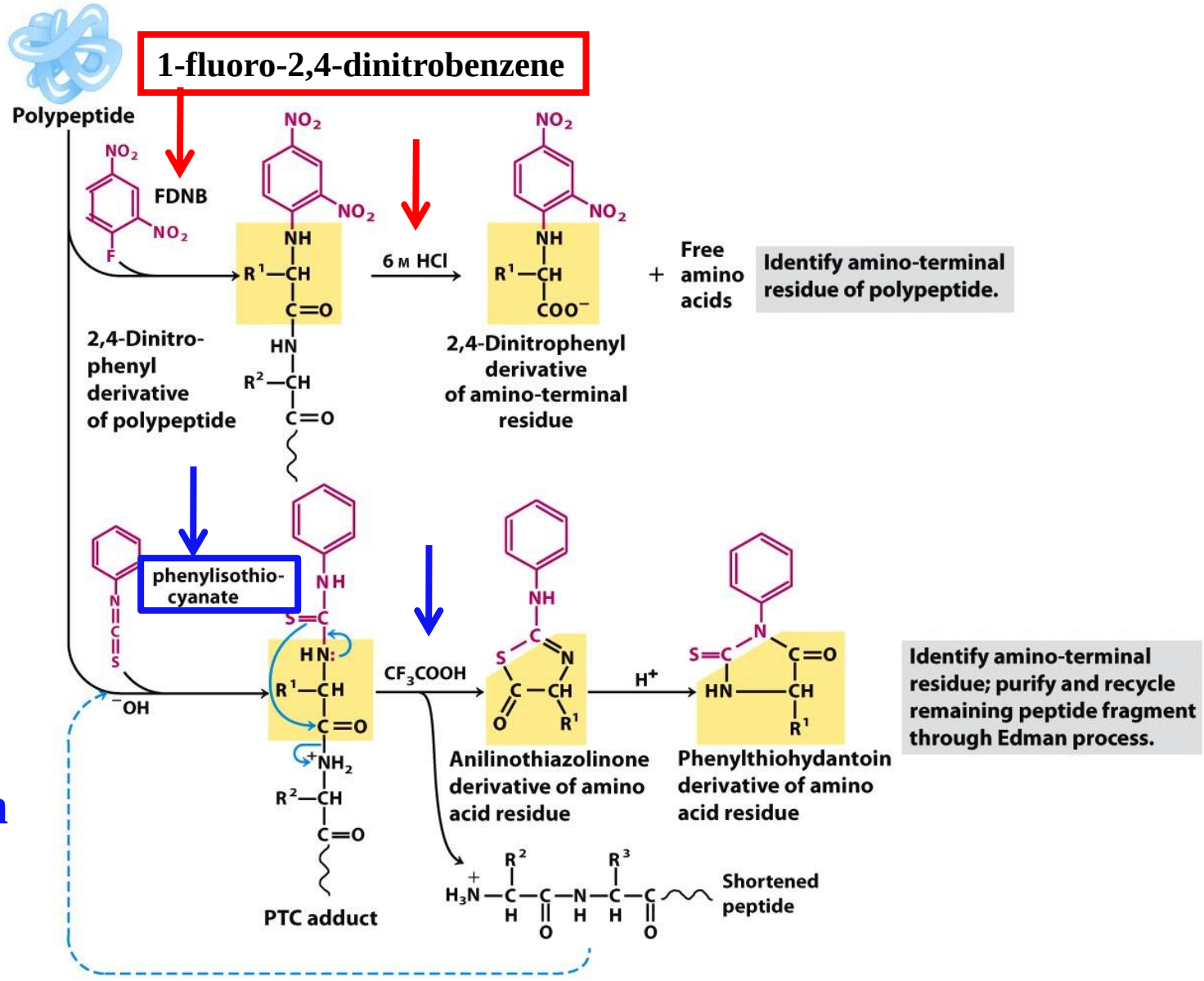


**Figure 3-25**
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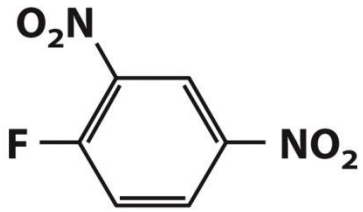
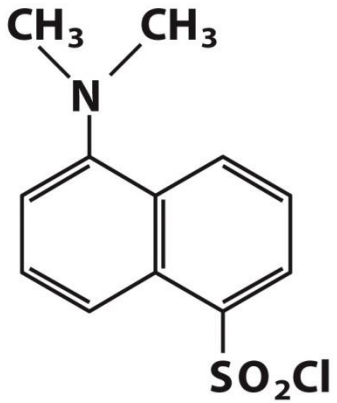
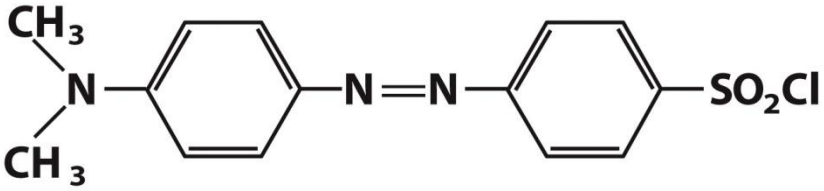



FDNB




Dansyl chloride




Dabsyl chloride

**Figure 3-26**
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# Reagents used to modify the α-amino group at the amino terminus

# Edman degradation

1. Label the N-terminal amino acid by PTH (phenylisothiocyanate)
2. Liberate the PTH-(N terminal) amino acid by mild acid, leaving an intact peptide shortened by one amino acid
3. Use chromatography to identify the labeled amino acid
4. Repeat step 1-3

Edman degradation can only be used to sequence peptides no longer than about 50 residues, because the efficiency of each step in Edman degradation is not 100% (e.g., $0.95^{50}=0.077$).

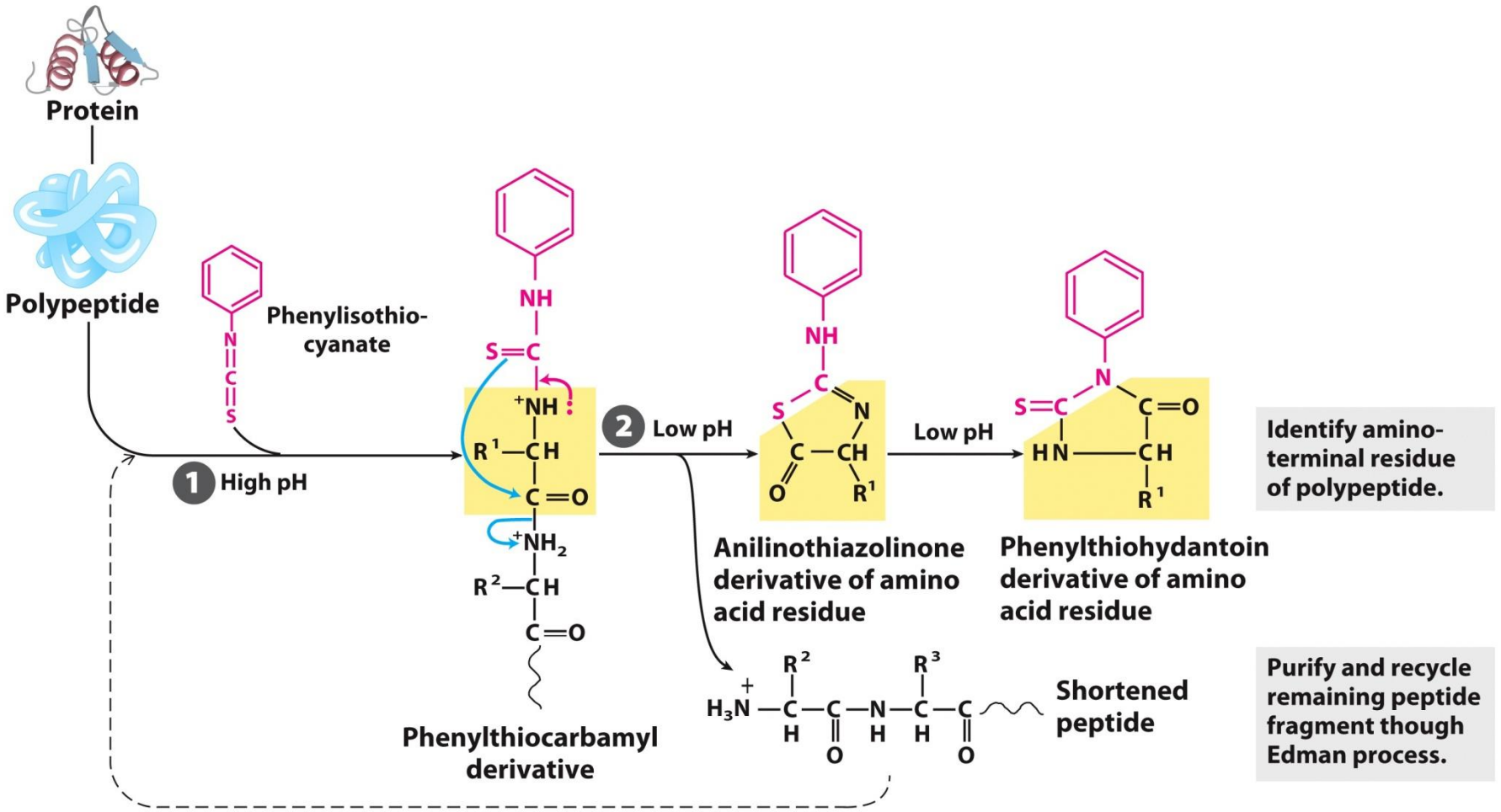


**Figure 3-27**
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# The protein sequencing chemistry devised by Pehr Edman

# Improved sequencing method

1. Cleave the original protein at specific amino acids into smaller peptides
2. Separate the peptides by chromatography
3. Sequence the small peptides
4. Order the peptides using information from overlap peptides

## TABLE 3–7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

*****

| Reagent (biological source)* | Cleavage points† |
|---|---|
| Trypsin (bovine pancreas) | Lys, Arg (C) |
| *Submaxillarus* protease (mouse submaxillary gland) | Arg (C) |
| Chymotrypsin (bovine pancreas) | Phe, Trp, Tyr (C) |
| *Staphylococcus aureus* V8 protease (bacterium *S. aureus*) | Asp, Glu (C) |
| Asp-*N*-protease (bacterium *Pseudomonas fragi*) | Asp, Glu (N) |
| Pepsin (porcine stomach) | Leu, Phe, Trp, Tyr (N) |
| Endoproteinase Lys C (bacterium *Lysobacter enzymogenes*) | Lys (C) |
| Cyanogen bromide | Met (C) |

*All reagents except cyanogen bromide are proteases. All are available from commercial sources.

†Residues furnishing the primary recognition point for the protease or reagent; peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.

Table 3-7

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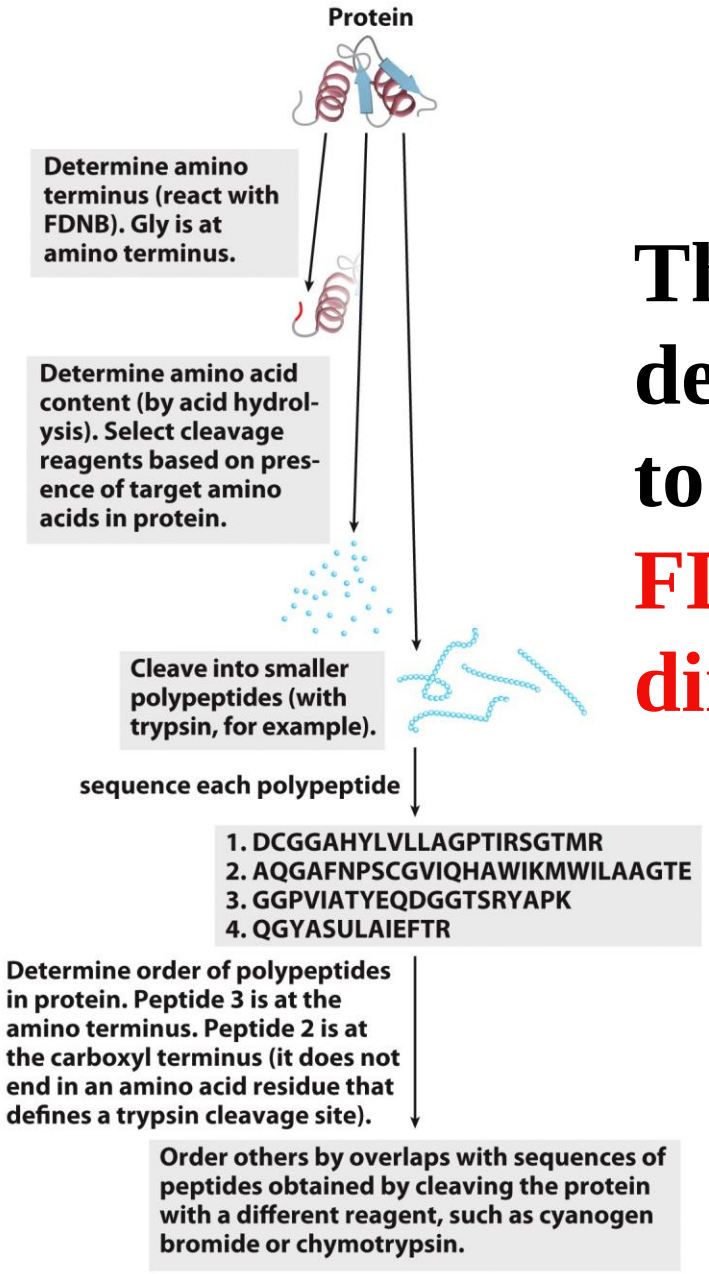


**Figure 3-25**
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**The procedures were developed by Fred Sanger to sequence insulin.**

**FDNB:1-fluoro-2,4-dinitrobenzene**

**Direct protein sequencing**

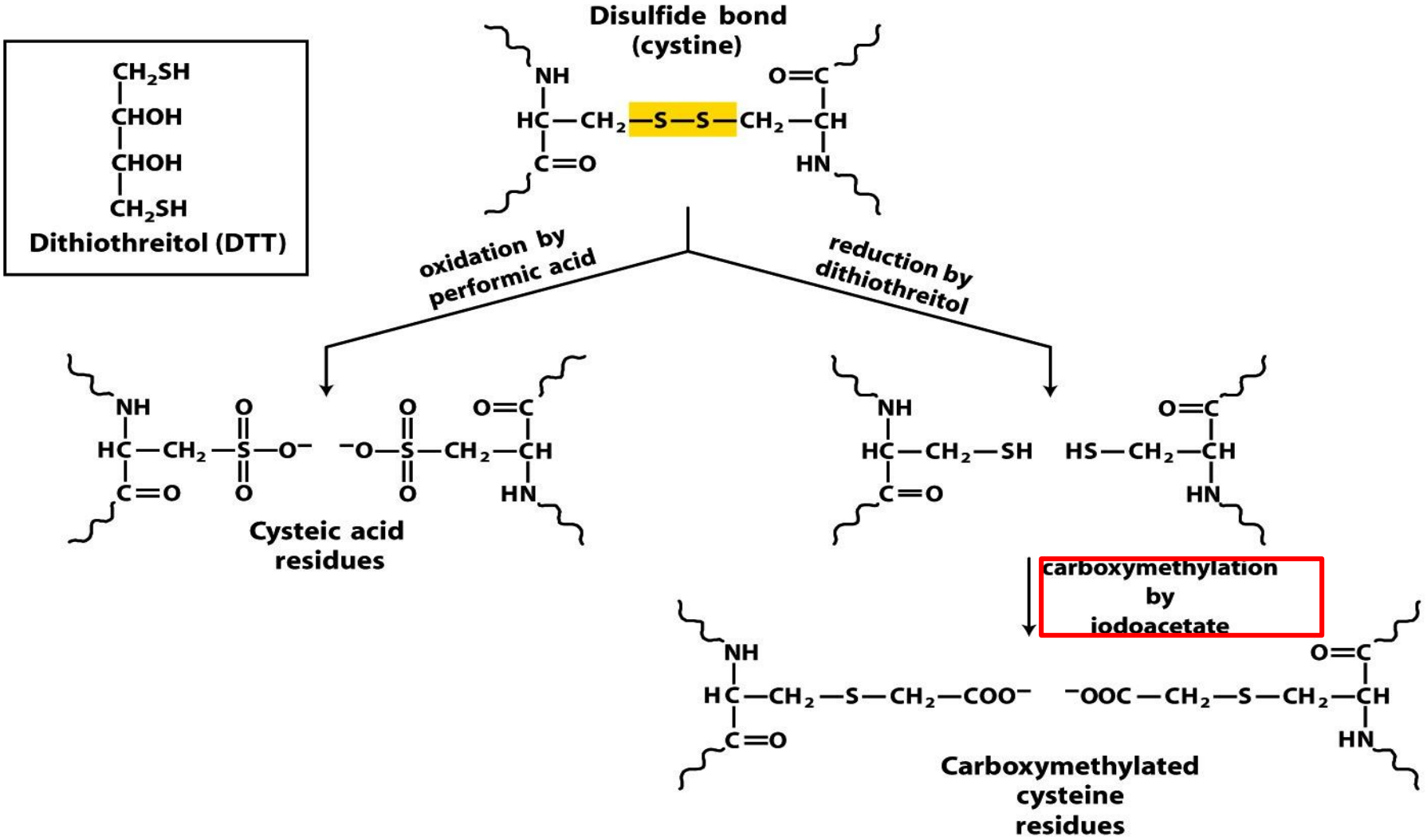


**Figure 3-26**
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# Breaking disulfide bonds in proteins

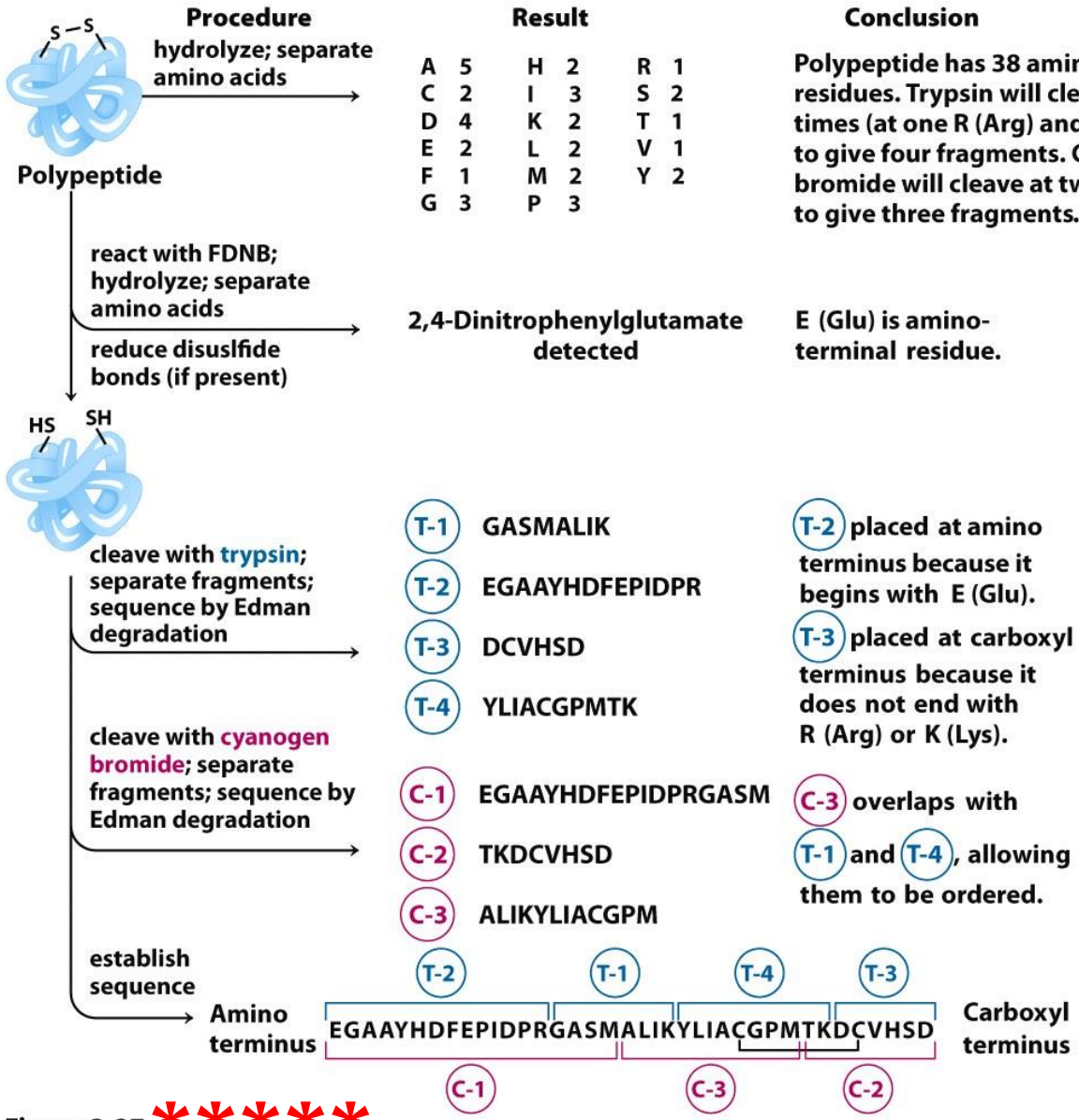


**Figure 3-27** *****

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# Cleaving proteins and sequencing and ordering the peptide fragments

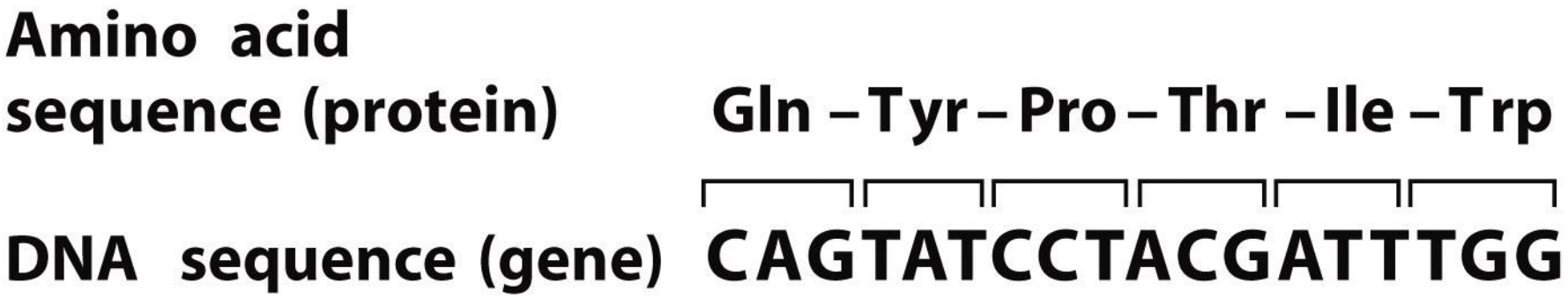


**Figure 3-28**
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# Correspondence of DNA and amino acid sequences

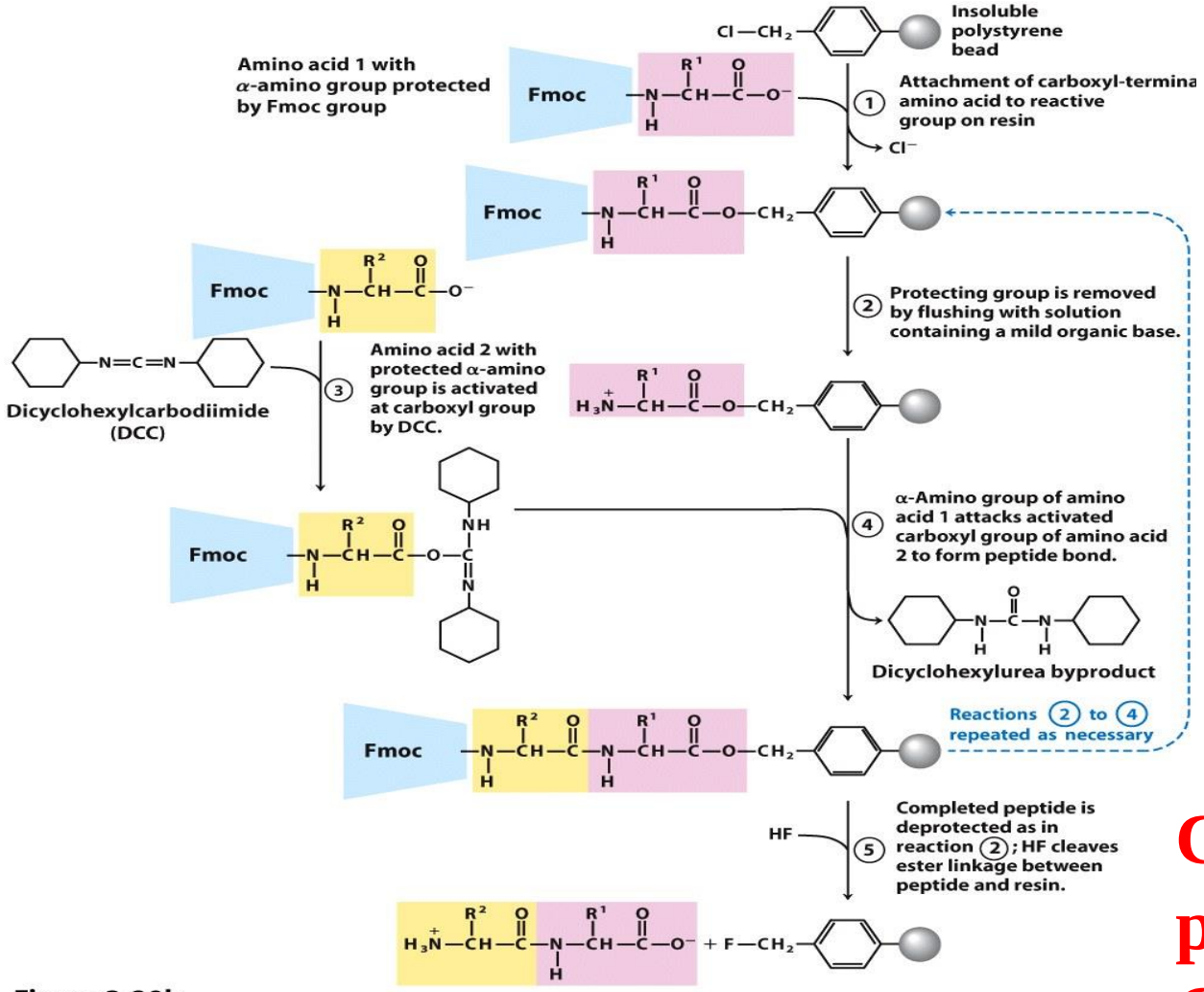


**Figure 3-29b**
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**Chemical synthesis proceeds from the C-terminus to the N-terminus!**

# Chemical synthesis of a peptide on an insoluble polymer support

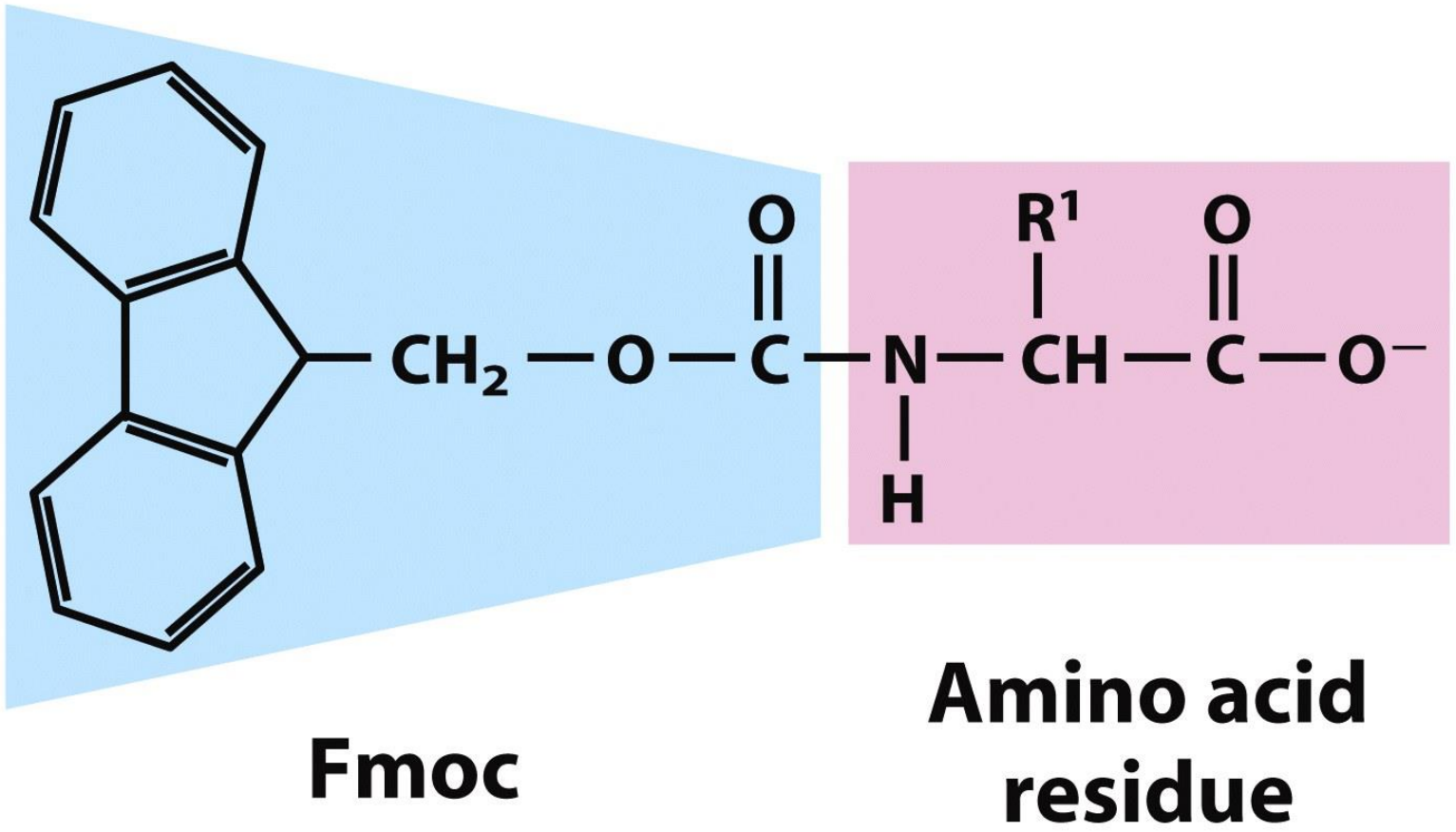


**Figure 3-29a**
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**The 9-fluorenylmethoxycarbonyl (Fmoc) group prevents unwanted reactions at the α-amino group of the residue**

# TABLE 3–8 Effect of Stepwise Yield on Overall Yield in Peptide Synthesis

| Number of residues in the final polypeptide | Overall yield of final peptide (%) when the yield of each step is: | |
|---|---|---|
| | 96.0% | 99.8% |
| 11 | 64 | 98 |
| 21 | 42 | 96 |
| 31 | 28 | 94 |
| 51 | 12 | 90 |
| 100 | 1.7 | 82 |

**Table 3-8**
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# Amino acid sequences provide important biochemical information

- **Knowledge of the amino acid sequence of a protein offers insights into its structure, function, cellular location, and evolution**
- **Consensus sequence: a sequence consisting of the residues that most commonly occur at each position in a set of similar sequences**
- **Homologous proteins (homologs): proteins having similar sequences and functions**
- **Paralogs: homologs present in the same species**
- **Orthologs: homologs present in different species**

# A comparison of sequences from different species reveals evolutionary relationship

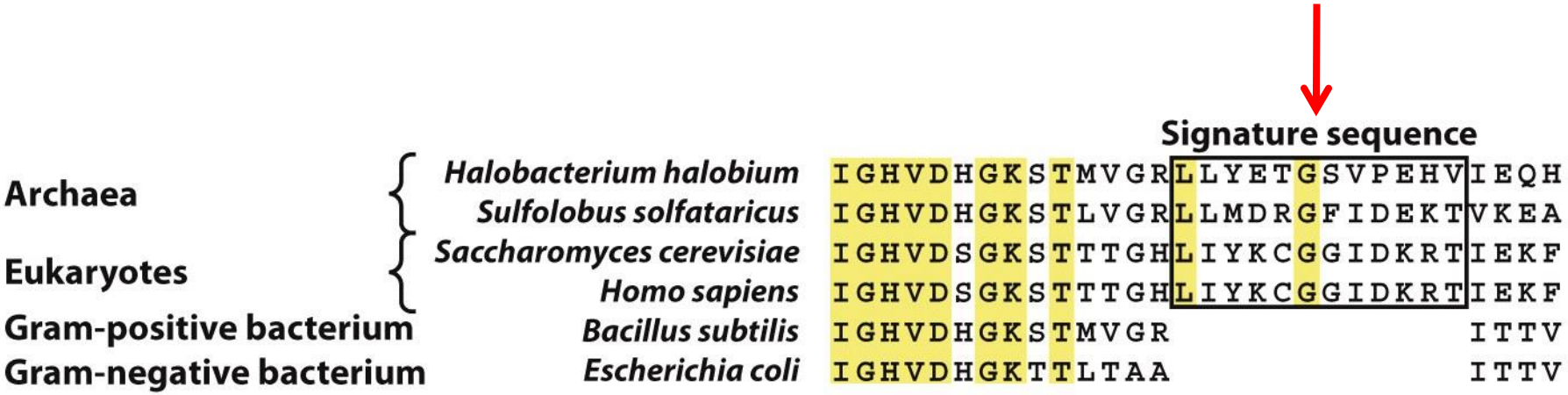


**Figure 3-31**
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# A signature sequence in the EF-1*a* /EF-Tu protein family

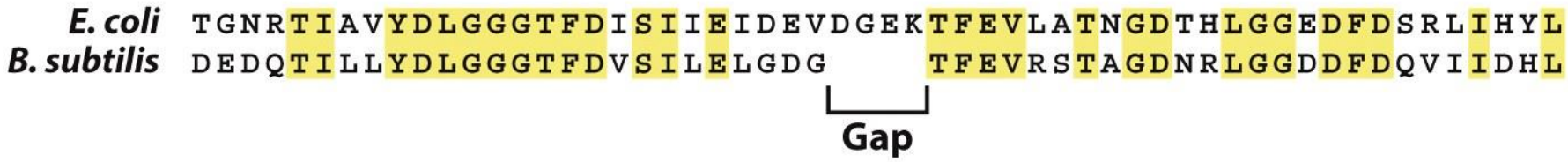


**Figure 3-30**
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# Aligning protein sequences with the use of gaps

# Useful website: www.ncbi.nlm.nih.gov

# Protein sequences as clues to evolutionary relationships

- **Sequences of proteins with identical functions from a wide range of species can be aligned and analyzed for differences.**
- **Differences indicate evolutionary divergences.**
- **Analysis of multiple protein families can indicate evolutionary relationships between organisms, ultimately the history of life on Earth.**

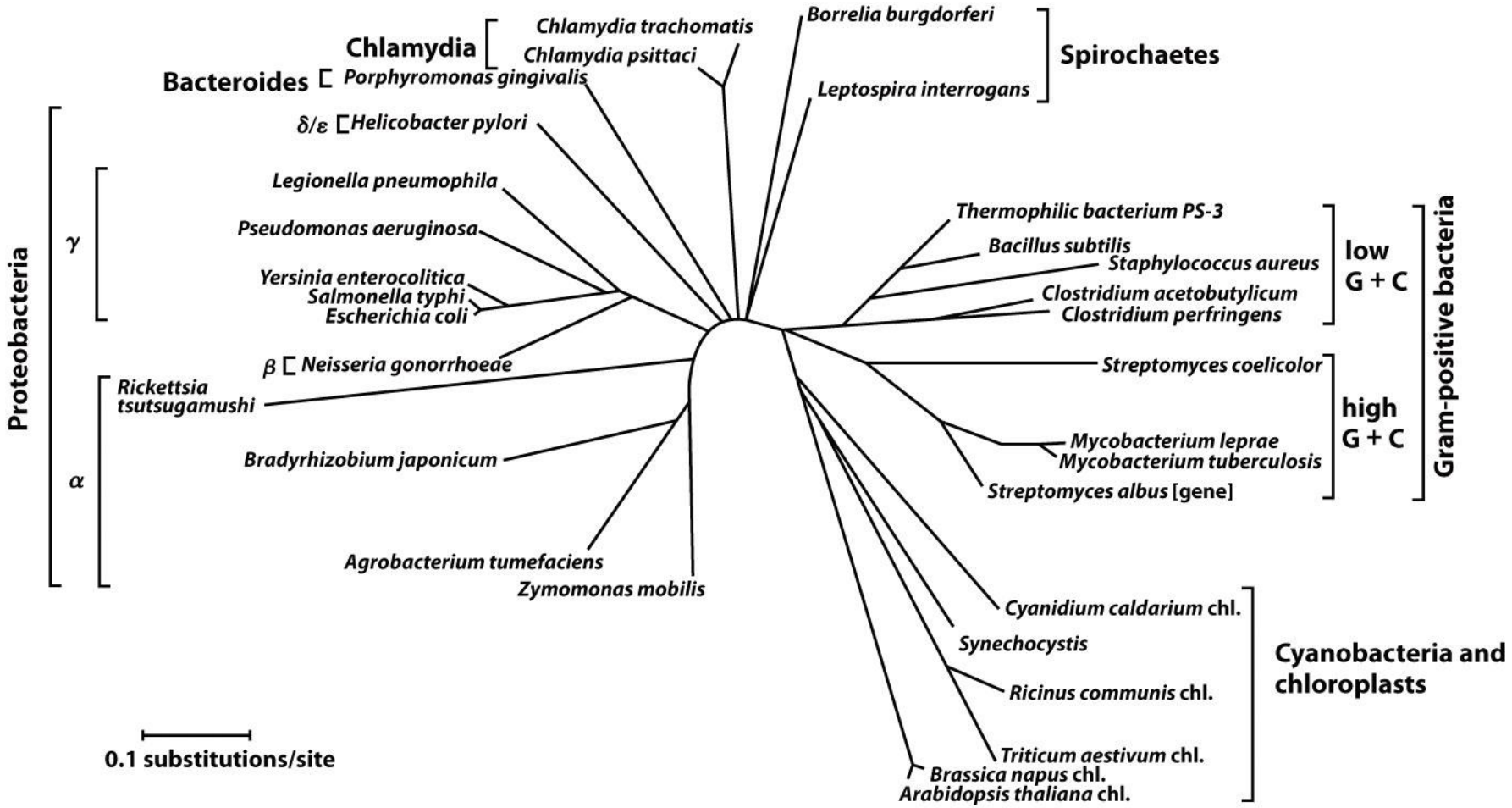


**Figure 3-32**
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## Evolutionary tree derived from amino acid sequence comparisons

**The basis for this tree is the sequence divergence observed in the GroEL family of proteins**

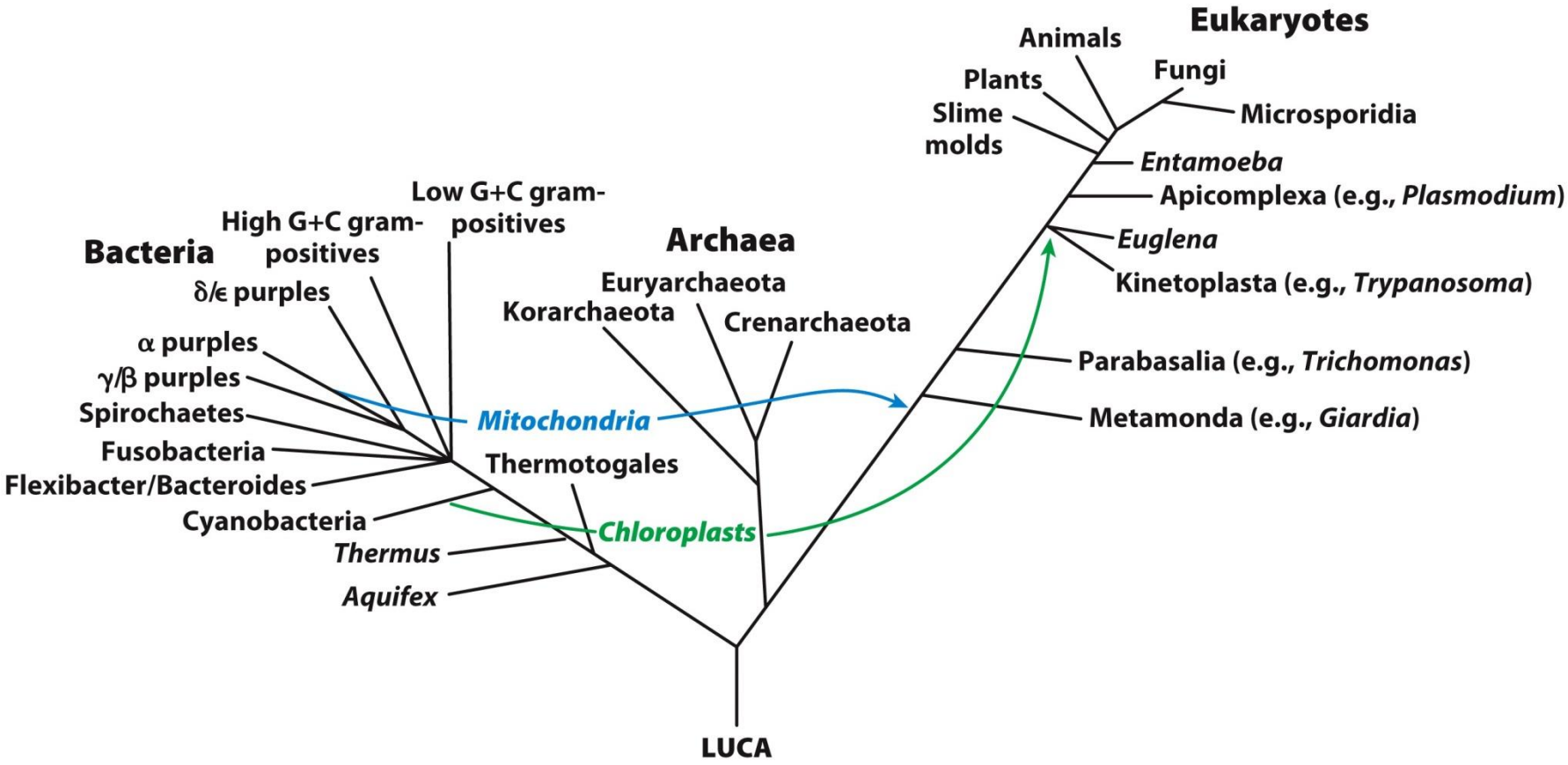


**Figure 3-36**
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# A consensus tree of life

**This tree is based on analyses of many different protein sequences and additional genomic features**

# Keywords

- **amino acid**
- **peptide**
- **protein**
- **pI**

# Words of the week

- **size**
- **charge**
- **solubility**
- **affinity**

# Summary

- **The amino acids in proteins are exclusively L stereoisomers.**
- **Amino acids can be classified into five main groups on the basis of the polarity and charge (at pH7) of their R groups.**
- **Amino acids can be joined covalently through peptide bonds to form peptides and proteins.**
- **Proteins can be separated and purified on the basis of differences in their physicochemical properties.**
- **Purification of proteins can be monitored by assaying specific activity.**